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- *Review Lecture (Übersichtsreferat)*
- *Abstracts (Kurzfassungen der Originalmitteilungen)*
- *Workshop contributions (Workshop-Beiträge)*
- *Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology (Mitteilungen des Ausschusses für Bedarfsnormen)*

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J. Hummel
Chairman

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1. Abstracts / Review Lecture / Workshop-Contributions:
Sciascia QL, De Leonardis D, Görs S, Metges CC 2025. The effect of glutamine supplementation on low birthweight suckling pig growth, organ mass and glutamine metabolism. Proc. Soc. Nutr. Physiol. 34:71.
2. Communications of the Committee for Requirement Standards:
GfE [Gesellschaft für Ernährungsphysiologie] 2025. Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology: Equations for estimating organic matter digestibility of compound feeds for ruminants. Proc. Soc. Nutr. Physiol. 34:139-144.

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09:55		86	Changes in intracellular carnitine levels and gene expression in bovine blood cells during lipopolysaccharide-induced inflammation and dietary L-carnitine supplementation **Seemann L., Kersten S., Bühler S., Billenkamp F., Meyer U., Visscher C., Huber K., Most E., Eder K., Dänicke S., Frahm J. — Brunswick / Hanover / Stuttgart-Hohenheim / Giessen*	115
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10:25		88	Preliminary data on the interaction of health status and diet zinc in piglets from Bavarian producers **Brugger D., Schiele D., Böttcher J., Dettmar L. — Zurich / Poing-Grub*	117

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		Workshop: Development and importance of the microbiome of the digestive tract of companion animals and livestock	
11:15		Moderation/Introduction *Hummel J. — Göttingen*	
11:25	WS1	Potential and pitfalls of microbiome research – What to consider? *Zwirzitz B.* — Vienna	129
12:00	WS2	Standardization in pig microbiome research to improve reproducibility and comparability *Camarinha Silva A.* — Stuttgart-Hohenheim	131
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Review Lecture

Sustainability of animal-sourced foods from a global perspective*

Nachhaltigkeit tierischer Lebensmittel im globalen Kontext

Qaim M. – Bonn

Introduction

Animal source foods (ASF) – including meat, dairy, eggs, and fish – are among the most contentious issues in the broader public debates about sustainable food systems, climate change, biodiversity loss, and healthy diets (Parlasca and Qaim 2022; Willett et al. 2019). This focus on ASF is not surprising, as their production and consumption are directly related to many key sustainability metrics, both positively and negatively.

In terms of positive contributions to sustainable development, ASF are rich sources of nutrients required for human nutrition, especially proteins, vitamins and minerals. Therefore, the consumption of ASF can help reduce widespread nutritional deficiencies and thus promote human health (Beal et al. 2024). The production of ASF also contributes to economic development, as the livestock and fisheries sectors are important sources of income and employment for more than one billion people worldwide, particularly in low- and middle-income countries (LMIC) (Adesogan et al. 2020). Livestock are often integrated into the mixed production systems of smallholder farmers. Another important role of the livestock sector is that animals, especially ruminants, are able to convert grass and crop residues that are inedible for humans into human food, thereby contributing to food and nutrition security on a planet with finite natural resources. Similarly, the fisheries sector converts both flora and fauna inedible to humans into human food.

In terms of negative contributions to sustainable development, most ASF have larger environmental and climate footprints than plant-based foods (Poore and Nemecek 2018). The livestock sector is a major driver of deforestation and global biodiversity loss, and accounts for a large share of agricultural greenhouse gas (GHG) emissions. Overfishing threatens the world's oceans and the functioning of marine ecosystems. In addition, depending on the production system, livestock and aquaculture can be associated with animal welfare and broader ethical concerns, although improvements have been made in some systems over time. Finally, intensive livestock production and consumption can be associated with human health issues, increasing the risk of air quality related health burdens, certain chronic diseases, and zoonoses (Qaim et al. 2024).

The multiple negative impacts of ASF are also the main reason why reductions in global livestock production and consumption are generally considered necessary to improve the sustainability of food systems (Willett et al. 2019). Some studies suggest that at least a 50% reduction in global ASF production and consumption would be required, while others calculate that even larger global reductions would be needed, especially for red meat, to stay within the planetary boundaries. Most studies acknowledge that regional differentiation is important because socioeconomic and environmental conditions can vary considerably (Qaim et al. 2024). Calls by some that vegetarian or vegan diets should be the goal for all people are not only unrealistic in the short to medium term, but also tend to neglect or ignore some of the trade-offs, as ASF also have notable positive effects.

Nevertheless, how to achieve more sustainable patterns of ASF production and consumption is an important consideration for research and policy-making and shall be briefly discussed in this paper. We review trends in ASF production and consumption, explore nutritional and environmental impacts, discuss technological options to reduce environmental footprints, and discuss options and constraints for dietary change towards more plant-based diets.

Trends in ASF production and consumption

While reductions in the production and consumption of ASF would be useful for food systems to remain within planetary boundaries, such reductions are not yet occurring. In fact, global production volumes of all types of ASF continue to increase substantially each year, in line with population and income growth, with no signs of slowing or declining (Fig. 1). For all types of ASF, the largest production growth is observed in Asia, which is predictable given Asia's remarkable demographic and economic development over the last decades.

This paper is a modified and abridged version of Qaim et al. (2024).

Fig. 2 shows the trends and levels of per capita consumption of ASF in various world regions. The differences are substantial, with people in high-income countries consuming significantly more than people in LMIC. In 2022, the average consumer in North America consumed about seven times more meat, milk, and eggs than the average consumer in Africa. Poor people tend to consume ASF relatively infrequently and in small quantities: due to income constraints, their diets are dominated by grains and other plant-based staples as the most affordable and accessible sources of food energy. But rising incomes lead to more diversified diets and higher consumption of ASF, a relationship that has been consistently observed in all world regions.

North America and Europe appear to have peaked in meat consumption, with mean per capita consumption now stagnating at high levels (Fig. 2A). For milk and eggs, mean per capita consumption in North America and Europe has still increased over the past 10 years (Fig. 2B, C). This is interesting because vegetarian and vegan lifestyles are becoming increasingly popular in some high-income communities. While this may be true for certain segments of the population, it is not true for the majority of the population and is not yet clearly reflected in aggregate consumption statistics. Only in Oceania have per capita consumption levels declined for most ASF.

In Africa, Asia, and Latin America, per capita consumption levels continue to rise along with the world average, underscoring that income growth, in addition to population growth, is another key driver of continued growth in global ASF production and consumption. It is not clear how this global trend could be reversed in the near future.

Nutritional effects of ASF

Despite these trends, malnutrition remains a major global problem. Close to 150 million children suffer from stunted growth, more than 700 million people are undernourished in terms of food energy, and about 3 billion people cannot afford a healthy diet that meets all their nutritional needs (FAO 2024). Poor people in LMIC are particularly affected by nutrient deficiencies. This situation is likely to be exacerbated by climate change, unless major adaptation and mitigation strategies are prioritized.

ASF contain essential macro- and micronutrients, helping to reduce nutritional deficiencies. Some micronutrients, such as vitamin B12, are unique to ASF. Others, such as iron, zinc, calcium, vitamin A, vitamin D, and omega-3 fatty acids, are found in higher amounts or in more bioavailable forms in ASF than in plant-based foods. A recent study with data from multiple countries in Africa shows that consumption of ASF significantly reduces the likelihood of child stunting (Fig. 3). These findings do not imply that ASF consumption should not be reduced where consumption quantities are high, but they clearly suggest that improving access to ASF for poor households in Africa could substantially help reduce child undernutrition. Other studies suggest that this is also true for poor households in Asia and elsewhere (Haile and Headey 2023).

Environmental effects of ASF

ASF have a much greater environmental impact than plant-based foods. While livestock production accounts for less than one-fifth of the total food energy consumed, it uses 70% of all agricultural land and 40% of arable land (Parlasca and Qaim 2022). Livestock also accounts for a disproportionate share of global freshwater use and up to two-thirds of all food-related GHG emissions. Further, livestock production is considered a major driver of global deforestation and biodiversity loss (Qaim et al. 2024).

Fig. 4 shows the environmental footprints of ASF and selected plant-based foods in terms of GHG emissions and land use per kg of food product. The mean results confirm that ASF have much larger climate footprints than almost all plant-based foods, even though with notable differences also within the group of ASF (Fig. 4A). Meat from ruminants is notably performing poorly because ruminants emit a substantial amount of methane, a very potent GHG. ASF also have larger land-use footprints than most plant-based foods, again with meat from ruminants having the most devastating impact (Fig. 4B). It is important to note that some of this land used by ruminants is grazing land that could not be used otherwise for food production. Nevertheless, negative effects on biodiversity and soils are widespread in heavily grazed regions. In addition, especially in some LMIC, grazing areas are increasingly expanded into forestlands (Qaim et al. 2024).

It is worth mentioning that environmental footprints cannot only be expressed per kg of food product, but also per unit of protein or other nutrients, which may be useful to better account for the nutritional values of ASF. However, the relative ranking of foods in terms of their environmental footprints is often unaffected by choosing alternative reference systems (Parlasca and Qaim 2022).

Role of production technology

What the mean environmental footprints shown in Fig. 4 conceal is that the environmental effects crucially depend on the production technology. For instance, the climate and land-use footprints of beef producers in the highest and lowest percentile worldwide can differ by a factor of more than 10 (Poore and Nemecek 2018). This is perhaps unsurprising given that cattle in Africa is held and managed under very different conditions than cattle in Europe or North America.

The larger part of the global livestock-related GHG emissions occurs in LMIC, mainly because of the lower relative productivity there, which results from traditional technologies and husbandry systems. In contrast, in high-income countries, more efficient systems have been developed that reduce the environmental footprint per unit of output. Given that the demand for ASF in LMIC will increase the most, the adoption of technologies to improve production efficiency there is key to both reducing livestock emissions and providing affordable access to ASF for improved human nutrition (van Eenennaam 2024).

Promising technologies in the livestock and fisheries sectors include improvements in animal management and health, genetics, and feeding practices. Animal management and health include aspects such as herd organization, grazing and manure management, and veterinary services that help improve animal productivity and reduce morbidity and mortality. Genetics includes the development and use of improved animal breeds. New genomic approaches can help increase the precision and speed of development of desirable animal traits related to productivity, fertility, disease resistance, etc. Improved animal feeding practices include the use of forages with higher digestibility and feed additives to reduce enteric methane emissions in ruminants. The use of circular feeds in livestock and fish production also has the potential to significantly reduce the environmental footprints of ASF. Circular feeds are low-cost feeds – such as crop and food by-products, residues and wastes – that cannot be consumed directly by humans. In some cases, the up-front use of fermentation processes or insect larvae can help extract nutrients from manure and other wastes, up-cycling valuable resources for circular food systems and bioeconomies (Qaim et al. 2024).

Convincing livestock and aquaculture farmers to use new types of environmentally-friendly inputs and technologies is easier when these innovations also help to increase productivity and profits, but may be more challenging when the private benefits for farmers are small. The successful implementation of such technologies will depend on governments providing the right incentive systems through fiscal or regulatory policies. In addition, private environmental standards set by large companies or industry associations can potentially play an important role.

In any case, there is widespread consensus that agricultural technologies and improved animal husbandry systems have an important role to play for making food systems more sustainable, but in addition to, not instead of, changing diets and consumption patterns.

Plant-based alternatives to ASF

Most nutrients and beneficial food compounds that the human body needs can be obtained from traditional plant-based foods, meaning that large quantities of ASF are not needed physiologically. For instance, legumes are particularly rich in protein, whereas vegetables, fruits, nuts, and seeds are good sources of micronutrients and essential fatty-acids. This is why people eating no or low quantities of ASF are especially advised to consume a variety of plant-based food groups in balanced proportions.

In addition to traditional plant-based foods, plant-based alternatives that try to mimic meat, dairy, egg, or fish products have recently become popular in some market segments. These meat, dairy, egg, or fish analogs are typically produced by food companies, targeting consumers who prefer "real" ASF but are willing to reduce consumption for ethical, environmental, or other reasons, yet without wanting to radically change the types of meals consumed (Qaim et al. 2024).

Mimicking the taste and texture of whole cuts of meat or fish is still technically difficult with existing technologies, so plant-based meat or fish analogs come mostly in the form of burgers (patties), nuggets, sausages, or crumbles (Pingali et al. 2023). For plant-based milk alternatives, various types – such as soy milk, oat milk, or almond milk – have gained some popularity. Food industry interest in plant-based meat and dairy alternatives already started in the late-1990s and early-2000s, but investments were scaled up especially during the last 10 years. The number of new manufacturers and brands in these sectors has skyrocketed since 2014 (Pingali et al. 2023). This is also reflected in high market growth rates of plant-based meat and milk analogs between 2014 and 2020, even though the growth has declined more recently in some market segments.

To provide some perspective: in 2020, 13 million tons of alternative protein products were consumed globally, which is less than 1% of the more than 1,500 million tons of combined global output of meat, milk, eggs, and fish. Nonetheless, further growth of plant-based alternatives is projected. Important to note is that so far developments are primarily concentrated in high-income countries. Around 80% of the currently existing alternative protein companies worldwide are located in high-income countries, 19% in middle-income countries (mostly upper middle-income), and none in low-income countries (van Eenennaam 2024). This is perhaps not surprising given that plant-based analogs are significantly more expensive than the ASF they try to mimic. For low-income consumers, such plant-based alternatives are currently unaffordable. Bringing down costs will be a major challenge for continued industry growth. Further improving taste, texture, and nutrient profiles will be other challenges to convince more consumers that these are good alternatives for ASF.

A recent study conducted a multi-criteria assessment of the sustainability of various meat and milk alternatives, looking at nutritional, health, environmental, and cost effects (Springmann 2024). Results suggest that traditional (unprocessed) plant-based foods perform best in terms of all criteria. Processed plant-based analogs perform somewhat worse, but still better than ASF in terms of most criteria, suggesting that replacing ASF with plant-based alternatives can result in multiple-win situations, at least in high-income countries.

Reducing ASF consumption and a stronger shift to plant-based foods will also require substantial behavioral change among consumers, which is difficult to achieve. Environmental labels and other types of information-related interventions may help to some extent. In addition, governments could incentivize more sustainable food choices through taxes and subsidies. However, taxes on meat and other ASF are unpopular and therefore hardly observed anywhere in the world up till now (Qaim et al. 2024).

Conclusion

ASF create important trade-offs that need to be managed through a mix of appropriate innovations, smart policies, efficient institutions, and behavior change. There are no one-size-fits-all blueprints. Rather, different transformation pathways need to be tailored to local conditions and constraints. In most LMIC, the production and consumption of ASF are likely to grow further. This is mostly good from a nutritional and human health perspective, but also poses environmental challenges. The adoption of improved technologies and pasture management strategies must be prioritized to reduce the environmental footprint of ASF.

In high-income and many upper-middle-income countries, average consumption of ASF should be reduced. Highly processed plant-based alternatives can support this transition to some extent, but they are still relatively expensive and need further technological and organoleptic improvements. In any case, consumption of more traditional, nutritious plant-based foods – such as legumes, vegetables and fruits – is even healthier and more sustainable, and should be the default option for replacing ASF. Overall, flexitarian diets with low to moderate consumption of dairy products and eggs and occasional consumption of meat and fish appear to be compatible with sustainable development.

Further research is needed in this important area, including the following questions: How can metrics of food system sustainability be improved for meaningful international comparison, covering heterogeneous conditions and multiple dimensions of sustainability? How can technologies to improve livestock productivity and reduce environmental externalities be implemented at scale, especially among smallholder farmers in LMIC, but also among livestock keepers in high-income countries? How can the supply of healthy, nutritious, and affordable food for all be improved, despite the challenges posed by climate change? What interventions can achieve major shifts toward more plant-based diets? How can efficient supply chains for alternative proteins be developed? This short article may hopefully draw attention to this important area and stimulate more interdisciplinary work on these and other questions related to sustainable food systems.

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Figures

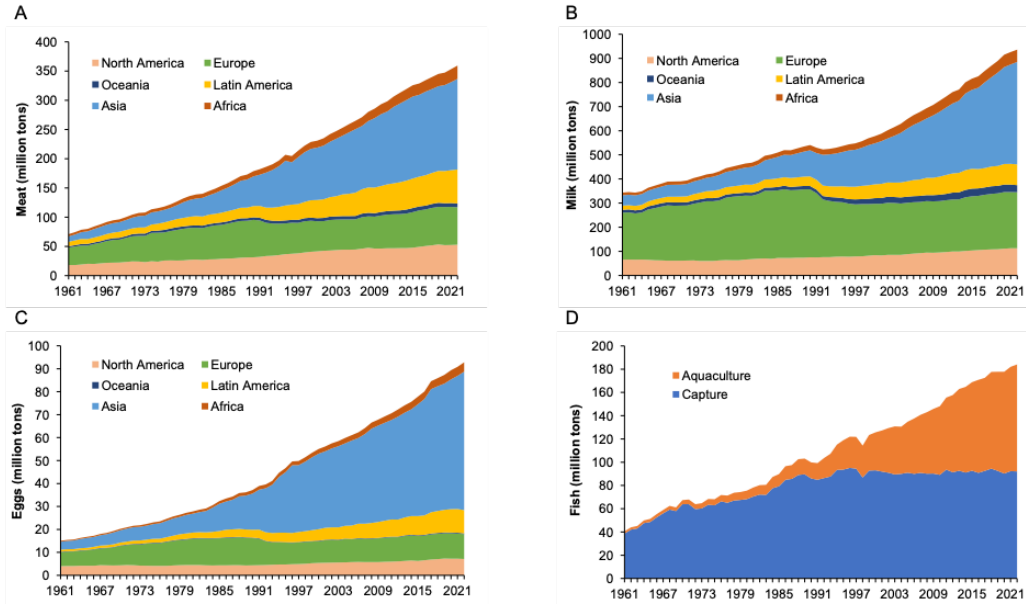

Fig. 1. Global production of animal-sourced foods (1961-2022). Based on data from FAO (FAOSTAT and GLOBEFISH 2024).

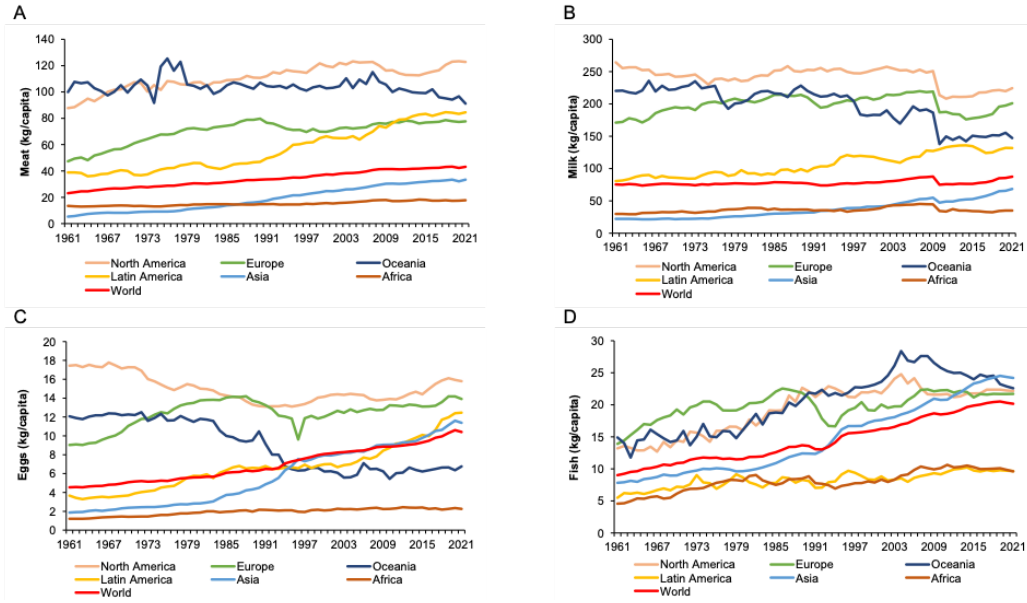

Fig. 2. Per-capita consumption of animal-sourced foods (1961-2022). Based on data from FAO Food Balances (2024). Note that FAO changed some of the Food Balances calculations, which explains the decline observed in 2010 for milk consumption in all regions.

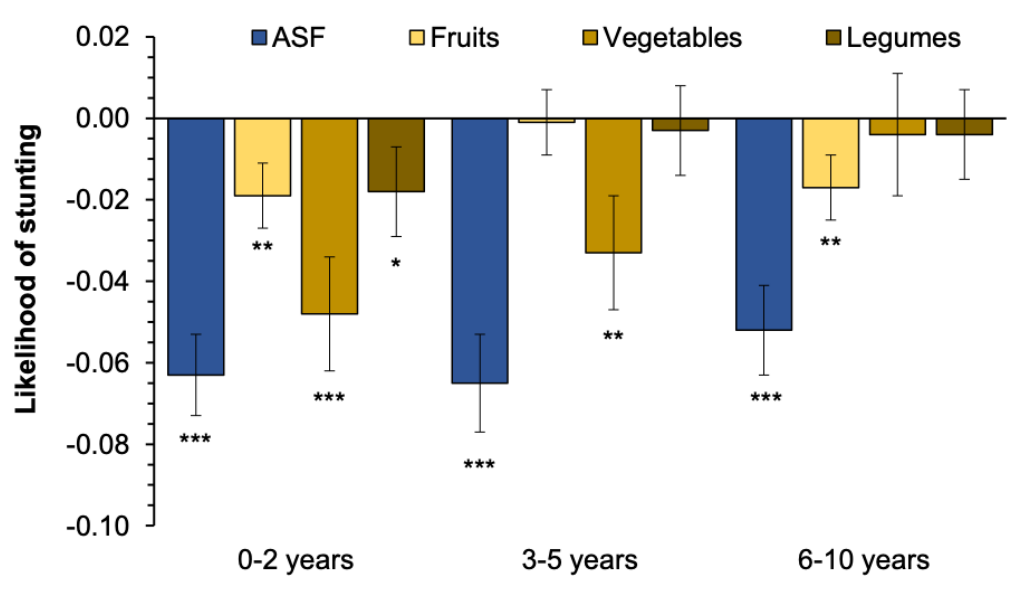

Fig. 3. Effects of animal-sourced foods (ASF) and other food groups on child stunting in Africa. Mean effects with standard error bars. *, **, *** indicate statistical significance at 10%, 5%, and 1% level, respectively. Source: Khonje and Qaim (2024).

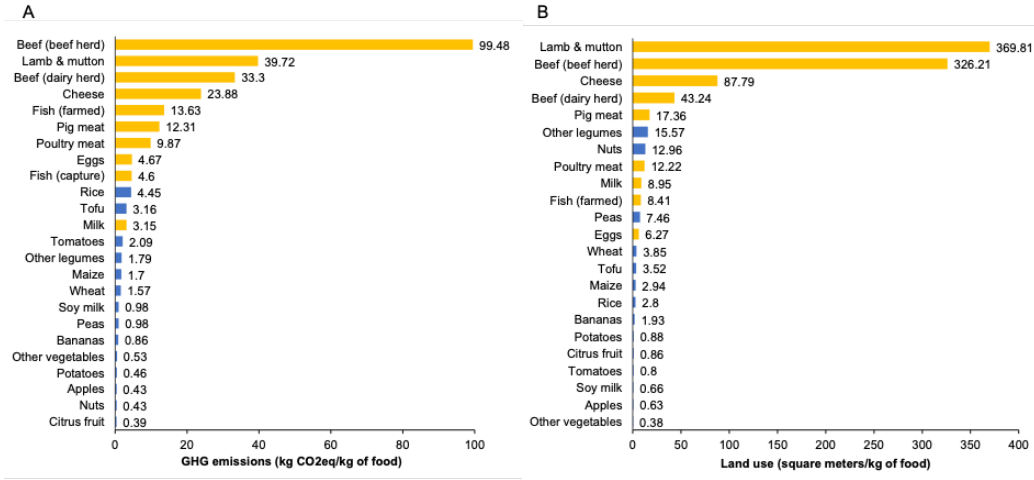

Fig. 4. Environmental footprints of animal-sourced and plant-based foods. Based on data from Poore and Nemecek (2018).

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Abstracts

1.

How is the exocrine pancreas function altered in a mouse model of pancreatic cancer?

Wie verändert sich die exokrine Pankreasfunktion in einem Mausmodell für das humane Pankreaskarzinom?

*Böswald L.F., Kohlmann L., Görgülü K., Popper B. – Planegg-Martinsried / Munich

In cancer research, genetically modified mouse models are used to mimic diseases relevant to human medicine. In our work, we characterize KCP mice that develop a form of pancreatic cancer based on their exocrine pancreatic functionality by determining the amylase activity at different points in the course of the tumour disease.

Methods: We used 32 mice of the line KCP (LSL-Kras^{G12D}; p53$^{F/F}$; Ptf1a-cre^{ex1}), including control mice (LSL-Kras^{G12D}; p53$^{F/F}$, abbreviated as CON). All mice were fed the same pelleted diet (23.5% crude protein, 4.2% crude fat, 6.2 % crude fiber, 25.1% starch, as-fed basis; degree of starch hydrolyzation 16 %) *ad libitum* (feed intake was not recorded). At defined time points (5 / 6 / 8 weeks of age), sub-groups of the mice of both genetics were sacrificed and dissected (number of mice per age group: 5 wks: 3 CON, 2 KCP; 6 wks: 12 CON, 12 KCP; 8 wks: 3 CON). Final body weight (BW) was recorded, including the weight of the pancreas and the filled, complete caecum. Amylase activity from pancreatic tissue and duodenal content was analysed immediately after sacrifice (Phadebas test kit). Blood glucose was tested with a handheld glucometer. The length of the intestinal tract (duodenum – rectum) was measured. Due to the uneven distribution of animal per age x genetic groups, a descriptive statistic was calculated, and only the 6-week-old mice were compared between CON and KCP via t-test (α=0.05).

Results: Final BW was lower in the KCP mice than the same-age CON mice. Blood glucose values showed a trend towards lower values in the KCP mice, especially the 6-week-old ones. Pancreas weight (% of BW) was significantly higher in the KCP mice than the CON mice (at 6 wks: CON: 0.92±0.27 % BW; KCP: 4.20±0.60 % BW; $p<0.0005$), caused by the tumour masses. In the CON mice, the amylase activity in the pancreas tissue increased with age. In the KCP mice, it was significantly lower than in the CON mice (at 6 wks: WT: 4602±2005 U/g; KCP: 1305±1727 U/g tissue; $p<0.001$), indicating the impairment of the normal exocrine function. The same was true for amylase activity measured in the duodenal content ($p<0.01$). Blood glucose showed a trend towards lower values in the KCP mice both at 5 and 6 weeks of age, which is in accordance with a lower capacity for enzymatic starch degradation and, thus, utilization. Total intestinal length (cm) tended to be lower in the KCP mice, especially in the 6-week-old ones (CON: 46±2.7; KCP: 40±3.5 cm; $p<0.0001$). Due to the lower amylase activity and resulting assumption of lower starch digestibility, a compensatory elongation of the intestine in the KCP might be expected, but the contrary was observed. This might be due to lower feed intake and nutrient utilization, contributing to an overall catabolic metabolic state and a resulting inability to compensate by intestinal elongation.

Conclusions: These results show the age-dependent increase in pancreatic amylase activity in mice, as known in other species but not yet investigated in mice. Furthermore, the reduced pancreatic amylase activity in the KCP mice seems to be correlated with disease progression, which may be relevant in clinical translational research (diagnosis, prognosis, clinical nutritional management). Further mice will be sampled to complement the groups (age, sex, genetics) and allow for more conclusive data analysis.

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2.

Fibre concentrations of hydrolysed peas affect gas production and volatile fatty acid composition in an *in vitro* model with pig faeces as inoculum

Fasergehalte hydrolysierter Erbsen beeinflussen die Gasproduktion und die flüchtigen Fettsäuren in einem in vitro-Modell mit Schweinekot als Inokulum

*Quinger F., Klein N., Camarinha-Silva A., Seifert J., Rodehutscord M. – Stuttgart-Hohenheim

Peas are a valuable source of energy and protein for pigs but also contain considerable amounts of carbohydrates that are not fully digested including α-galactosides and non-starch polysaccharides [1]. Precaecally undigested carbohydrates can be fermented in the hindgut, with volatile fatty acids (VFA) as major end products. We hypothesised that the fermentation characteristics of *in vitro* hydrolysed pea residues would differ between different pea varieties.

Methods: Twelve different pea varieties were hydrolysed in two steps with pepsin at pH 2 and pancreatin at pH 6.8 using the assay of Boisen and Fernandez [2]. The hydrolysis residue was lyophilized and used as a substrate in the modified Hohenheim gas test [3] with pig faeces as inoculum to study microbial fermentation. Four growing barrows fed commercial feed and without medication were used as donor animals. 200 mg of each hydrolysis residue was transferred into a glass syringe and incubated with 30 mL of a buffer/faeces mixture at 39°C in 6 separate runs. Gas production was recorded at 0, 2, 4, 6, 8, 12, 24, 36, and 48 h, and samples for VFA analysis were taken at 8 and 24 h. An exponential equation was fitted to the gas production data using a nonlinear mixed effects model with the pea variety as a fixed effect and the batch, incubator, and syringe as random effects in the nlme package of R. Potential gas production and the rate of gas production were compared with a Wald test. Concentrations of VFA were compared using a linear mixed effects model with the pea variety as a fixed effect and the batch, incubator, and syringe as random effects using the lmerTest package. Pairwise post-hoc comparisons were computed using the emmeans package. Pearson correlation coefficients between gas production, VFA concentration, and analysed nutrient concentrations of the hydrolysis residue were calculated for the non-tannic pea varieties (n = 11) and P-values were controlled for multiple testing by the Benjamini-Hochberg procedure.

Results: The disappearance of nutrients by *in vitro* hydrolysation was on average 87, 98, 44, and 9 % for dry matter (DM), crude protein (CP), total dietary fibre (TDF), and neutral detergent fibre (aNDFom), respectively. Concentrations of CP, TDF, aNDFom, and acid detergent fibre (ADFom) in hydrolysis residues ranged from 27 to 64, 733 to 989, 521 to 714, and 422 to 602 g/kg DM, respectively. Potential gas production and gas production rate differed among hydrolysis residues ($P < 0.05$) and ranged from 49 to 77 mL/200 mg DM and 4.7 to 9.3 %/h, respectively. Positive correlations were detected between TDF, aNDFom, and ADFom concentrations in the hydrolysis residues and the potential gas production ($P < 0.05$). Branched-chain fatty acids concentrations after 24 h were negatively correlated with TDF, aNDFom, and ADFom concentrations in hydrolysis residues ($P < 0.05$).

Conclusions: The residues of different *in vitro* hydrolysed pea varieties vary markedly in their fermentability *in vitro*. The correlations imply that fibre fractions in the residue may cause differences in gas production and reduce the formation of branched-chain fatty acids from amino acids. Thus, the fibre content of peas, which is not hydrolysed by the end of the ileum, may alter protein fermentation in the hindgut of pigs.

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3.

Effect of mineral phosphorus in the feed on mucosal phosphatases and their hydrolysis products in laying hens at two ages

Auswirkungen von mineralischem Phosphor im Futter auf die mukosalen Phosphatasen und deren Hydrolyseprodukte in unterschiedlich alten Legehennen

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Chickens can degrade some of the phytate (InsP6) contained in feed by their mucosal phosphatases. Little is known about the characteristics and degradation products of these phosphatases. The activity of mucosal phosphatases has been determined in broilers and turkeys by enriching the brush border membrane (BBM) of the intestinal mucosa, followed by incubation with sodium phytate and measurement of released phosphate (Pi) [1]. However, studying the degradation products besides Pi with this assay is impossible. To characterise mucosal phosphatase activities based on the InsP6 degradation products, a three-step *in vitro* digestion assay with a feed phytate matrix [2] was modified for the purpose of this study by adding BBM to the system. The aim was to consider the *in vitro* degradation products and the enzyme activity data together for a comprehensive characterisation of the mucosal phosphatases of laying hens of two different strains fed two different diets and sampled at two different ages, i.e., before and after the onset of laying activity.

Methods: The experiment implied a 2×2×2-factorial arrangement of treatments with the factors hen strain (Lohman Brown-classic (LB) and Lohman LSL-classic (LSL)), hen age (week 19 and 24), and mineral P supplement (0 (P-) and 1 (P+) g P/kg feed) [3]. The diets were fed for 4 weeks. In weeks 19 and 24 of age, 10 hens in each treatment were sacrificed, duodenum mucosa was obtained, and BBM was enriched [1]. The three-step *in vitro* assay simulated crop, stomachs, and small intestine at 40°C, considering digestive enzymes and the pH value. A mix of maize and soybean meal was used as the phytate matrix and contained 11.0 µmol $InsP_6$/g, 0.6 µmol Ins(1,2,4,5,6)P_5/g and 0.2 µmol Ins(1,2,3,4,5)P_5/g. At the beginning of the small intestine step, 1600 µg BBM protein was added to the system. The incubation was followed by InsP extraction and chromatographic analysis. Controls without added mucosa were implemented. Each sample was run at least in duplicate. In a second assay, the phosphatase activity of BBM was directly determined with 25 µg sodium phytate at pH 5.5 for 15 min at 40 °C [1]. Data were analysed in a 3-factorial analysis of variance using the MIXED procedure of SAS.

Results: There were no significant three- or two-way interactions of the factors on the *in vitro* InsP6 disappearance during the incubation and concentrations of InsP isomers in the incubation residue. InsP6 disappearance was significantly lower in 19-week-old than in 24-week-old hens ($P < 0.001$). The concentrations of Ins(1,2,3,4,5)P_5, Ins(1,2,3,4,6)P_5 and Ins(1,2,3,4)P_4 in the incubation residue were significantly lower ($P \leq 0.002$), and those of $InsP_3$ and lower phosphorylated InsP were significantly higher ($P \leq 0.027$) when BBM of 24-week-old hens was used compared to 19-week-old hens. The mucosal phosphatase activity of the BBM was also affected by hen age only ($P < 0.002$). It was by 0.8 µmol Pi/g BBM protein/min lower in 19-week-old than 24-week-old hens.

Conclusions: The measured isomers suggest a 5- and 6-phytase activity in the duodenal mucosa. The results of both assays, with higher phosphatase activity and a higher InsP degradation in 24-week-old hens, are consistent and provide a comprehensive characterisation of these enzymes. Added mineral P in the feed did not affect the mucosal enzymes under the conditions of this study.

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4.

Effect of dietary mineral P renunciation on NaPi-IIb and TRPV6 protein expression in duodenal enterocytes of two high-performing laying hen strains before and after start of egg laying

Auswirkungen eines Verzichts auf mineralischen P auf die Proteinexpression von NaPi-IIbund TRPV6 in duodenalen Enterozyten zweier hochleistender Legehennenlinien vor und nach Beginn des Eierlegens

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Active intestinal phosphate (Pi) transport in chicken is mainly mediated by NaPi-IIb (sodium-dependent Pi transporter of the solute carrier family SLC34), which expression in apical membranes of enterocytes can be modulated by luminal Pi content and bird age [1,2]. This study aimed to investigate NaPi-IIb protein expression in the duodenum in response to P-renunciated diet in two strains of laying hens before and after the onset of egg laying. Furthermore, given that variations in luminal P concentrations may also influence the transport of ionized calcium (Ca^{2+}) [1], the expression of transient receptor potential vanilloid cation channel, subfamily V, member 6 (TRPV6), which mediates the Ca^{2+}entry into the enterocytes across the brush border membrane [1, 2] was also assessed.

Methods: The experiment followed a 2×2×2 factorial design, with treatments based on three factors: hen strain (Lohmann Brown-Classic (LB), Lohmann LSL-Classic (LSL)), period (weeks 19, 24), and dietary mineral P supplementation (0 g P/kg feed (P-), 1 g P/kg feed (P^+)). The layer diets were calculated to contain 3.1 or 4.1 g P/kg, 1.3 or 2.3 g NPP/kg, and 35 g Ca/kg. After 4 weeks of treatment feeding, at 19 and 24 weeks of age, 10 hens from each group were sacrificed. The concentration of Ca and Pwas assessed in digesta (duodenum+jejunum) and blood plasma [3]. Duodenal mucosa samples were collected, and brush border membranes (BBM) were enriched by $MgCl_2$-precipitation method. Western Blot was performed as previously described [1]. Membranes were blocked with 5% milk/PBS, 0.1% Tween for 2 hours, and incubated overnight at 4°C with primary antibodies against NaPi-IIb, 1:1000 (N0035-26C, USBiological) and TRPV6, 1:2000 (ACC-036, Alomone Labs). Detection was performed using 1:2000 anti-rabbit HRP-conjugated secondary antibodies (7074P2, Cell Signaling Technology) and chemiluminescent substrate (Biozym). Protein expression was semi quantified by densitometry (Quantity One Software, BioRad). Signal intensity was normalized to protein content per lane as indicated by Indian ink staining. The specificity of NaPi-IIb antibodies was confirmed by pre-incubation with antigenic peptide (N0035-26B, USBiological), the TRPV6 antibody was a commercially provided chicken-specific antibody. Data were analysed using a 3-factorial analysis of variance with the MIXED procedure of SAS.

Results: Dietary P levels did not affect protein expression of both, NaPi-IIb and TRPV6. However, NaPi-IIb protein expression increased from 19 to 24 weeks in LSL hens (P = 0.012), while LB hens showed no changes across age periods (interaction period*strain P = 0.040). TRPV6 expression exhibited no age-related differences in LSL hens, whereas LB hens tended to reduce its expression from 19 to 24 weeks (interaction period*strain P = 0.074). For both hen strains, the P and Ca concentrations in the duodenum+jejunum significantly decreased from 19 to 24 weeks when fed P^+. The plasma Pi concentration remained stable, while plasma Calevel strongly increased at 24 weeks, especially when fed P^+ (interaction period*P P = 0.003) [3].

Conclusions: The results demonstrate different mechanisms of maintaining mineral homeostasis in hens of different strains. In response to lower content of P in duodenum+jejunum there was an increase in NaPi-IIb expression in LSL hens, which suggests a compensatory reaction and might lead to more efficient P absorption. Notably, the downregulation of TRPV6 in the LB strain at 24 weeks, despite a significant rise in plasma Ca levels, may indicate that LB hens rely more on other Ca absorption mechanisms in the intestine like passive paracellular Ca transport through the tight junctions due to the electrochemical potential difference.

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5.

Effects of a phytogenic feed additive (PFA) on the performance and apparent prececal nutrient digestibility of growing broilers

Wirkungen eines phytogenen Futterzusatzes (PFA) auf Leistungsparameter und die präzäkale Nährstoffverdaulichkeit bei wachsenden Broilern

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The use of antibiotic growth promoters in livestock feeding is meanwhile banned in the European Union since 2006. Numerous alternatives have been evaluated, including phytogenic substances. The phenolic essential oils Cinnamaldehyde, Thymol and Eugenol induce the Nrf2-pathway which in turn up-regulates the gene expression of major intestinal amino acid- and small peptide-transporters [1]. Saponins increase gene expression- and membrane association of amino acid- and small peptide transporters like PEPT1 [2]. Capsaicin stimulates the intestinal heat- and pungent-sensitive Ca-channel TRPV1, followed by serotonin release and the secretion of pancreatic digestive enzymes [3]. Consequently, the aim of the present study was to test the efficacy of a combination of encapsulated essential oils (Cinnamaldehyde, Thymol, Eugenol), Capsaicin, and mixed triterpene- and steroid saponins from quillaja, yucca and fenugreek on growth performance and prececal nutrient digestibility of growing broilers.

Methods: Thirty six one day old Cobb500 broiler chicken with a mean body weight of 43.9±1.30 g were randomly assigned to the experimental groups Con (no additive) and PFA (phytogenic feed additive) and placed into 12 pens. Thus, both groups included 6 repetitions with 3 birds each. The birds were fed a commercial broiler starter diet for 21 days. The PFA was prepared, using spray-chilling encapsulation with hydrogenated rapeseed oil and contained 20% essential oils (each 6,67% Cinnamaldehyde, Thymol and Eugenol), 0.15% Capsaicin, and 3.5% mixed triterpene- and steroid saponins from quillaja, yucca and fenugreek. No additive (Con) or the PFA (100 mg/kg diet) were added to the diets. Moreover, diets of both groups contained 3000 mg/kg titanium-IV-oxide as an inert digestibility marker. The trial was approved by the local animal welfare authorities of Berlin (A 0439/17). Performance was evaluated at days 7, 14 and 21 by recording feed intake, body weight and the calculated feed conversion ratio (FCR). On day 21 broilers were euthanized. Subsequently, digesta samples were collected from the posterior half between Meckel's diverticulum and 2 cm cranial to the osteum ileocecale and pooled from the 3 birds of a pen. Digesta were freeze-dried and analyzed for dry matter,- energy,- fat,- protein,- ash,- calcium (Ca),- phosphorus (P)- and titanium concentration. Apparent prececal nutrient digestibility was defined as the quotient of percentual nutrient content in ileum and feed, corrected by the respective abundance of indigestible titanium. After ensurance of normality of distribution, all performance- and nutrient digestibility-parameters were analysed by unpaired Tukeys t-test.

Results: During the entire experimental period, statistically significant differences ($p<0.05$) occurred for body weight gain (991±8.41 vs. 1006 ±8.93g) and FCR (1.267±0.029 vs. 1.244±0.025 g/g) between groups Con and PFA. Apparent prececal digestibility of dry matter (57.18±1.34 vs. 57.53±1.83 %), crude fat (86.24±2.33 vs. 85.17±1.35 %) and starch (94.13±0.38 vs. 94.45±0.31 %) was not increased by the PFA. In contrast, addition of the PFA to diets improved apparent prececal digestibility of protein (79.96±0.71 vs. 81.80±1.30 %), ash (44.22±1.42 vs. 48.73. ±1.42 %), Ca (45.61±1.43 vs. 50.04±1.47 %) and P (54.20±1.58 vs. 59.17±1.16 %) highly significant ($p<0.01$) compared to Con broilers. In summary the PFA turned out as efficient performance enhancer for weight gain (1.51±0.094%), FCR (-2.00±0.048%), prececal protein,-Ca- and P-digestibility (2.25±0.10%, 8.86±1.50%, 9.09±1.37%), respectively.

Conclusions: The present study has demonstrated that PFA can play an important role as non-antibiotic growth promoters, and their efficacy largely depends on the selection and combination of single phytogenic ingredients. The particular strong effects of the tested PFA on apparent prececal protein,- ash,- Ca- and P-digestibility can be explained by the influence of the ingredients on the above mentioned physiological pathways.

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6.

Evaluation of Vitamin D, its Precursors, and Biofortification through UV-B Exposure in Insect meals

Untersuchung von Vitamin D, seiner Vorstufen und dessen Biofortifizierung mittels UV-B Exposition in Insektenmehlen

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Vitamin D is essential for animal health and is traditionally supplemented in animal nutrition due to low concentrations in natural feed sources. Studies indicate that edible insects, including larvae of *Hermetia illucens* (black soldier fly) and *Tenebrio molitor* (meal beetle), offer the potential for vitamin D biofortification by UV-B exposition [1, 2]. This study aimed to (1) identify and quantify vitamin D precursors and metabolites in processed insect larval meals, (2) enhance the vitamin D content of these meals via UV-B exposure, and (3) evaluate the bioefficacy of insect-derived vitamin D *in vivo*.

Methods: Full-fat *T. molitor* (97.8% DM) and partially defatted *H. illucens* meal (89.9% DM) were used in this study. Insect meals were analysed for vitamin D_2, D_3, and their precursors, ergosterol and 7-dehydrocholesterol (7-DHC), using HPLC. Meals were exposed to UV-B light (290–310 nm) for varying exposition times (7.5, 15, 30 and 45 min) and spreading densities (6.3, 12.6, 25.2, and 50.4 mg/cm²). A feeding study was conducted with 12 groups of 6 rats, each fed different diets, varying in vitamin D forms (D_2 or D_3), sources (pure vitamin D or UV-B exposed insect meal) and concentrations (1500, 2250, or 3000 IU per kg diet). Serum 25(OH)D metabolites were quantified by HPLC-MS/MS. Data were analysed separately for dietary vitamin D_2 and D_3 sources using a two-way ANOVA with vitamin D source and concentration as factors, followed by Tukey‘s post-hoc test. Significance was set at $P < 0.05$ for all analyses. Data are given as means ± SD.

Results: In *H. illucens* meal the concentration of ergosterol was 252 ± 15 µg /g, while 7-DHC was not detectable. *T. molitor* meal contained 530 ± 7 µg 7-DHC/g, and 50.9 ± 3.0 µg ergosterol/g. Prior to UV-B exposure, *H. illucens* contained 0.302 ± 0.054 µg D_3/g and no detectable D_2, while *T. molitor* showed no detectable vitamin D_2 or D_3. Upon UV-B exposure, the formation of vitamin D_2 in *H. illucens* and vitamin D_3 in *T. molitor* was significantly affected by both exposure time and spreading density. Increasing the spreading density from 6.3 to 50.4 mg/cm² decreased the formation of D_2 in *H. illucens* by 74% and the formation of D_3 in *T. molitor* by 34%. Longer UV-B exposure times resulted in a linear increase in vitamin D formation, with maximum concentrations of 13.8 ± 0.9 µg D_2/g in *H. illucens* and 34.4 ± 1.8 µg D_3/g in *T. molitor* observed (UV-B exposure 45 min, spreading density of 12.6 mg/cm²). *H. illucens* meal also showed a slight increase in vitamin D_3 concentration during UV-B exposition. In the feeding study, rats supplemented with UV-B-exposed *H. illucens* meals showed a dose-dependent increase in serum 25(OH)D_2 concentrations (in nmol/l, 1500 IU: 23.7 ± 2.2, 2250 IU: 31.0 ± 3.1, 3000 IU: 31.3 ± 4.0), which were significantly higher ($P = 0.007$) than in the groups receiving pure vitamin D_2 (in nmol/l, 1500 IU: 21.1 ± 3.6, 2250 IU: 26.7 ± 1.6, 3000 IU: 28.9 ± 3.02). Similarly, UV-B-exposed *T. molitor* meals resulted in a greater increase in serum 25(OH)D_3 (in nmol/l, 1500 IU: 36.9 ± 5.5, 2250 IU: 52.7 ± 9.8, 3000 IU: 78.3 ± 22.1) compared to pure vitamin D_3 (in nmol/l, 1500 IU: 28.1 ± 3.9, 2250 IU: 38.6 ± 8.4, 3000 IU: 57.1 ± 20) ($P = 0.004$), along with detectable concentrations of 25(OH)D_2.

Conclusions: This study shows that *H. illucens* meal is rich in the vitamin D_2 precursor ergosterol, whereas *T. molitor* meal is rich in the vitamin D_3 precursor 7-DHC. Exposure to UV-B light effectively increased the concentrations of vitamin D_2 in *H. illucens* meal and vitamin D_3 in *T. molitor* meal, with the formation being influenced by spreading density and UV-B exposure time. Diets supplemented with UV-B exposed insect meals resulted in significantly higher serum concentrations of 25(OH)D metabolites than those with equivalent amounts of pure vitamin D_2 or D_3, indicating a greater bioefficiency of insect meal-derived vitamin D. Overall, UV-B-exposed insect meals present a novel, highly bioavailable vitamin D source for animal nutrition.

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7.

Amino acid and fatty acid patterns of mineral-enriched black soldier fly larvae through supplementation of sewage sludge recyclates

Aminosäure- und Fettsäuremuster von Larven der Schwarzen Soldatenfliege, die aufgrund einer Supplementierung mit Klärschlamm-Rezyklaten einen erhöhten Mineraliengehalt aufweisen

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The larvae of the black soldier fly (BSFL; *Hermetia illucens*) are efficient in converting low-grade biomass into valuable protein. We have shown that BSFL accumulate minerals from waste biomass such as sewage sludge recyclates [1]. Although feeding with waste is prohibited under current feed legislation, upcycling through BSFL can play a role in the future of circular agriculture. Supplementing sewage sludge recyclates (SSR), namely single superphosphate (SSP) or biochar (BCH), resulted in different mineral accumulation (e.g., Ca, P, Fe, Cd) and was found to affect the protein and lipid content of BSFL [1]. We hypothesized that the amino acid (AA) and fatty acid (FA) patterns are differentially affected by the supplementation of different SSR sources.

Methods: Newly hatched BSFL were fed with chicken starter feed (CF) until day (d) 5. Then they were separated from CF, counted and weighed. Based on their weight, an estimated number of 8000 larvae were spread to 40 x 60 cm crates filled with 10 kg wet experimental feed (water to feed ratio = 70:30 (w/w)). The larvae were batch fed at d 5 (2.5 kg), d 9 (4 kg), and d 13 (3.5 kg). The diets were based on a modified standard fly diet (Gainesville diet, GD) used as control [1], containing wheat bran, corn meal, alfalfa hay and dried sugar beet pulp (15.4 % crude protein, 10.9 MJ gross energy (GE) / kg dry matter (DM), 7.9 g Ca and 4.8 g P / kg DM). The GD was supplemented with 3.6% SSP (GD+SSP) or 4% BCH (GD+BCH) at the expense of 2% wheat bran and corn meal (14.7 % crude protein, 10.2 MJ GE/kg DM). Harvesting was done after the first occurrence of prepupae at d 18 to d 20 of development. The freeze-dried BSFL (n = 6 crates per diet within 2 runs) were analysed for their AA (after acidic hydrolysis) and FA pattern using HPLC and GC, respectively. Data on AA and FA were analysed using the GLM procedure of SAS considering the treatment effects plus blocking effects of experimental runs. Multiple comparisons of groups were done by the Tukey test ($P < 0.05$).

Results: Most AA were least concentrated in the GD+SSP group (13 out of 16 AA). The AA pattern of the GD+SSP differed significantly compared to the GD group in 7 of 16 AA. Highest AA concentrations were found in the GD group. The contents of glutamic acid plus glutamine was highest in all groups (mean 41 mg / kg DM). The most concentrated essential amino acid in the GD group was leucine (26 mg / kg DM). Methionine showed the lowest level within all groups (mean 5 mg / kg DM). The lysine level was relatively high in all groups (mean 21 mg / kg DM), with a lysine to total AA ratio of 0.07. Especially the saturated fatty acids (SFA) were affected by the SSR supplements (6 of 15 measured SFA). Numerically, the contents in the GD+BCH group were lower than in the GD+SSP group and significant e.g., for pentadecanoic acid (C15:0), stearic acid (C18:0), eicosanoic acid (C20:0). Lauric acid (C12:0) accounted for the largest percentage of all FA (ca. 41%). The proportion of the mono-unsaturated oleic acid (C18:1 n 9c) was about 11% in all groups. The poly-unsaturated fatty acid (PUFA) linoleic acid (C18:2 n 6c) showed the highest proportions among all PUFAs in the GD+BCH group (14%); its percentage was lowest in the GD+SSP group (11%). The conjugated linoleic acids (cis-9, trans-11 CLA plus trans-10, cis-12 CLA) were numerically highest in the GD group (0.3%), compared to the GD+SSP and GD+BCH group (0.2%) ($P = 0.3$).

Conclusions: The patterns of AA and FA in BSFL were differentially affected by the two SSR supplements. Minerals that influence AA and FA pattern in BSFL individually or in their combination found in SSP or BCH, need to be identified to understand their interaction with AA and FA metabolism and their impact on protein and fat quality of BSFL supplemented with SSR or mineral mixtures.

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8.

Impact of varying amounts of mealworm meal or poultry by-product meal in a diet on immunological parameters of healthy adult dogs

Einfluss variierender Gehalte an Mehlwurmmehl oder Geflügelmehl im Futter auf immunologische Parameter von gesunden adulten Hunden

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An insect-based diet is an interesting dietetic option for dogs affected by a food allergy, as it often represents a novel protein source for the organism [1]. On the other hand, edible insects can also induce allergies, as described in human medicine [2,3]. Owing to the clinical relevance, it was therefore the aim of the present study to evaluate the immunological effects of mealworm meal in a diet for dogs at different dietary inclusion levels.

Methods: Ten healthy adult beagle dogs received four experimental diets. The diets contained poultry by-product meal or mealworm meal at a moderate protein level (3.66 % nitrogen (N) and 3.47 % N in dry matter (DM), respectively) or at a high protein level (5.17 % N and 5.45 % N in DM) and were offered to all dogs in a randomized cross over-design. The general condition, feed acceptance and fecal score of the animals were recorded daily. Blood samples were collected at the beginning of the last week of each 4-week feeding period. The blood leukocytes were isolated and intended for phenotyping, mitogen-induced proliferation assays and phagocytosis assays, using flow cytometry. Plasma immunoglobulin (Ig) G, M, A and E concentrations were measured with commercial ELISA kits. Data were analyzed with SAS 9.4 and SPSS 28, taking also missing values into account. A repeated measures ANOVA and Bonferroni corrected pairwise comparisons were conducted to evaluate the dietary effects of the protein source, protein level and their interaction. The statistical significance was set at $P < 0.05$.

Results: Four dogs refused the moderate-protein diet including poultry by-product meal and were removed from the respective feeding period. The corresponding diet with mealworm meal and the high-protein diets were well accepted by all dogs. No gastrointestinal disorders were observed in the animals throughout the study. The immunological data revealed a decrease of $CD4^+$ (T helper) cells ($P = 0.0073$) and an increase of $CD8^+$ (cytotoxic T) cells ($P = 0.0438$) in the blood, when the insect-based diets were fed. A higher protein level also decreased the CD 4^+ ($P = 0.0192$) and increased the $CD8^+$ cells ($P = 0.0178$). The dietary treatment had no impact on the proliferative activity of peripheral blood mononuclear cells ($P > 0.05$). An interaction effect of the dietary protein source and level could be detected for the phagocytic activity of blood granulocytes ($P = 0.0399$) and the Ig A ($P = 0.0425$) and IgE concentrations in the plasma of the dogs ($P = 0.0438$), which could, however, not further specified by the pairwise comparisons ($P > 0.05$).

Conclusions: The dietary protein source and level partly affected the immune function of the dogs. However, as no health problems were observed in the different feeding groups, the clinical relevance of these findings seems to be small. Nevertheless, the immunological and dietetic properties of insect-based diets should be further evaluated in diseased animals with immunological dysregulations.

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9.

Effects of mixed microalgae or parts of mint plants from an aquaponics system on growth performance of black soldier fly larvae

Effekte von Mikroalgen und Pflanzenteilen von Minze aus einem Aquaponic System als Supplemente im Futtersubstrat für Larven der Schwarzen Soldatenfliege

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The larvae of the black soldier fly (BSFL; *Hermetia illucens*) have a high capacity to convert low-grade biomass into valuable protein. Plant (e.g. mint) cultivation with process water from fish production in aquaponics systems and algae reactors reduce the mineral and nitrogen load of the process water. Only mint leaves can be marketed. The unused parts of mint and the biomass produced by the algae reactor can be used in part as a substitute for feed substrates for larvae such as wheat bran, which can also be used for other farm animals. The use of microalgae mixtures and plant roots produced with process water is prohibited by feed legislation, but its use could be important to close nutrient cycles in the future. Recently, it was shown that growth of BSFL can be improved when their substrate is supplemented with extracts from microalgae [1]. We hypothesized that feeding BSFL with a standard Gainesville fly diet (GD) supplemented with algae or mint parts reared with process water would provide at least comparable growth performance to feeding the larvae with GD alone.

Methods: Newly hatched BSFL were fed with a chicken starter feed (CF) until day (d) 5. Then they were separated from the residual substrate, counted and weighed. A number of 150 larvae was spread to 500 ml insect growth vessels (n = 6 per diet). These were filled with 114 g of the GD diet composed of 30% lucerne, 20% maize meal, 50% wheat bran as control (15.9% crude protein (CP), 12.8 MJ gross energy (GE) / kg dry matter (DM)) or GD supplemented with 10%, 20% or 30% dried microalgae (18.8% CP, 7.5 MJ GE / kg DM) produced by an algae reactor employing wastewater of a recirculating carp aquaculture system, at the expense of wheat bran. In addition, the GD diet was supplemented with 10% or 20% mint leaves (30.9% CP, 9.5 MJ GE / kg DM), mint stem (12.3% CP, 4.4 MJ GE / kg DM) or mint root (20.0% CP, 7.6 MJ GE / kg DM) (the latter 7.3% instead of 10%) at the expense of wheat bran. The specific growth rate (SGR) was calculated as follows: SGR = ((ln (body weight in g at harvest (market body weight)) – ln (initial body weight in g))/ fattening period (d)) × 100 [2]. Effects of algae and mint treatments were analysed separately using the GLM procedure of SAS. Multiple comparisons of treatment groups were done by the Tukey test ($P < 0.05$). In addition, pooled samples of larvae per diet were analysed for dry matter and carbon to nitrogen ratio (C/N ratio).

Results: The algae supplementation of 10% and 30% had no effect on larval growth performance compared to the GD diet, but was lower with the 20% algae supplement than with all other groups. The SGR of the larvae when using the algae supplement at any concentration was lower compared to the GD larvae. The mint leaves had no effect on the growth performance of the larvae, nor did 10% mint roots. All other supplements led to lower performance for total and individual larval mass. The SGR was higher in larvae reared with GD as well as with 10% mint leaves or 20% mint roots compared to all other mint groups. For algae supplementation, the larvae DM was between 27.2% (GD) and 30.4% (20% algae), whereby a C/N ratio of 5.7 vs. 6.0 was determined, respectively. For mint supplementation, the DM of BSFL was between 24.4% (10% mint stem) and 30.9% (20% mint leaves) associated with a C/N ratio of 5.5 vs. 6.3.

Conclusions: To summarize, the microalgae grown on process water can be used in part to replace feed substrates, e.g. wheat bran, for larvae. Stems and roots of mint reduced the growth performance of the larvae, while the effect of the leaves was comparable to the pure GD control diet but changes of the SGR and therefore the BSFL rearing time should be considered. Our results indicate that although mint oil is used as an insect repellent, the addition of up to 20% mint leaves or mint by-products do not affect the growth performance of BSFL.

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10.

How does source and treatment affect mineral and trace element content in drinking water for laboratory rodents?

Wie beeinflusst der Ursprung und die Behandlung den Gehalt an Mengen- und Spurenelementen im Trinkwasser für Versuchsnager?

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In laboratory animal science, knowledge about diet and nutrient intake is important. Water must also be considered as a source of elements, especially since facilities use differently treated water sources. In this study, we aimed to compare the mineral and trace element content of drinking water used for lab animals and to quantify the importance in terms of nutrient intake.

Methods: Samples of drinking water for laboratory rodents from two facilities in the UK and twelve in Germany (DE) were collected. Mineral and trace element concentration (n=31 elements measurable) was determined by inductively-coupled plasma mass spectrometry (ICP-MS) with LGC-Seronorm L2 Urine as the certified reference material. Comparisons between water source/treatment and area were conducted for the most important elements (t-tests/Kruskal-Wallis, α=0.05).

Results: In total, 36 DE samples (12 of these were tap water, 2 acidified, 15 purified, 4 sterile filtrated and 3 mixed) and 24 UK samples (6 of these were tap water, 13 were filtered and 5 purified) were collected Many elements in tap water varied markedly between areas, despite similar source, e.g. calcium (all values median [min; max]; UK samples 9.41 [5.58; 49.72] mg/L; DE samples: 74.51 [0.23; 87.86] mg/L; $p<0.01$), magnesium (UK samples 2.15 [1.48; 9.57] mg/L; DE samples: 18.64 [0.01; 21.25] mg/L; $p<0.01$) and selenium (UK samples 3.11 [0.69; 6.58] µg/L; DE samples: 0.16 [0.08; 0.26] µg/L; $p<0.005$). The same was true for different types of water offered within each institution. For example, acidified water had greater P (48.12 mg/L vs. <LOD in other samples), purified water, as expected, had few elements above LOD, whereas filtered water appeared to remove Na, whilst adding S. It needs to be investitaged how the variation of water mineral content may contribute to the variation in total daily nutrient consumption (from feed and water) and how this may influence mineral homeostasis and acid-base-balance, among other things. The lead (Pb) concentration was considerable in tap water and differed significantly between origins. Indeed, five samples exceeded the target limit of 5 µg/L given in the directive (EU) 2020/2184 for drinking water (currently, the transition limit of 10 µg/L is valid).

Conclusions: We have shown for the first time, that drinking water as a source of minerals is a potential source of high variation for lab animals. Potential effects on the animals themselves and on reproductive performance including transgenerational inheritance cannot be ruled out [1,2,3].

Acknowledgement: We are grateful for all facilities providing water samples for this study.

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11.

Relation between feeding, management and bodyweight in clucking dual-purpose hens of Coffee origin

Zusammenhang zwischen Körpermasse, Fütterungs- und Haltungsmanagement bei brütigen Zweinutzungshennen der Herkunft Coffee

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In dual purpose hens, clucking results in reduced egg production and bodyweight loss due to less time spent feeding. This study aimed to investigate if placing clucking hens next to the feeder influences their bodyweight and broodiness and to what extent a correlation between these factors can be established.

Methods: 270 Coffee hens (ÖTZ, 29-63 weeks old) were kept according to the Regulation (EU) 2018/848 on organic production and fed a complete diet (10.4 AME_N MJ; 15.3% XP; 0.28% methionine). Hens were included in the study if clucking in the nest was performed for at least one week. During brooding, the hens were allocated to one of three treatment groups: positioning next to the feed at least once a day (feeding), housing in a separate clucking compartment (housing) or no treatment (control). Bodyweight (BW) was measured weekly. The statistical analysis was carried out using t-test, Pearson correlation, one-way ANOVA and post-hoc Bonferroni ($p<0.05$).

Results: 119 clucking periods from 71 hens were analysed. The average duration was 30 (7-91) d with differences between repetitions (1st: 38.0±19.1 d, 2^{nd}: 32.4±16.8 d; 3^{rd}: 26.3±1.9 d). The average BW before clucking was 2.5 kg and 2.3 kg at the end ($p<0.001$) with a positive correlation between the duration of the brooding period and BW loss ($r=0.555$; $p<0.001$). On average, the hens lost 9.7 g (0.38%) of their initial BW/d. The daily BW loss correlated positively with the initial weight ($r = 0.266$; $p=0.003$). The treatment „feeding“ resulted in an average BW loss of 7.97 g/d, hens from treatment „housing“ lost 2.35 g BW/d and control animals lost 10.47 g BW/d ($p<0.01$).

Conclusions: Placing broody hens next to the feed or in a different compartment can help to reduce bodyweight loss.

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12.

Formic acid addition to legume silages: methods of dry matter correction

Zugabe von Ameisensäure zu legumen Silagen: Methoden der Trockensubstanzkorrektur

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Producing stable, protein- and riboflavin-rich silage with low dry matter (DM) content for pig fattening is a challenge. Legumes, especially vetches, are difficult to ensile due to their low fermentation coefficient. The pH value can be reduced to values between 3.9 and 4.4 by adding the chemical additive formic acid. Good professional practice stipulates that the DM content of silage should be corrected so that it is not underestimated [1][2]. The corrections currently used are based on the chemical analysis of volatile ingredients, but do not take into account the addition of chemical silage additives [2]. The extension of the chemical analysis and the addition of the FA concentration to the DM correction should not be neglected. First of all, the addition of FA reduces the formation of fermentation products and furthermore, FA has a high volatility coefficient in the drying process. The higher use of formic acid in vetch silages influences the DM content and thus the DM correction.

Methods: The forage of three vetch species, each with two varieties, was harvested for an ensiling trial in 2022 (*Vicia villosa*: cv. Latigo and Villana, *Vicia pannonica*: cv. Beta and Detenicka, *Vicia sativa*: cv. Carbure and Rubis). Four field replicates were set up for each harvesting stage of vetch (bud stage, flowering stage, pod formation stage and pod filling stage) with two 1 L jars each (n=96). The silage was prepared with the silage additives buffered formic acid (Amasil®NA (10 ml kg^{-1} FM); Dr. Pieper Technologie- und Produktentwicklung GmbH) and lactic acid bacteria (BIO SIL® (1,2 mg kg-1 FM); Dr. Pieper Technologie- und Produktentwicklung GmbH). The ensiling duration was 90 days. Determination of DM content was calculated after drying the biomass to constant weight (24 h, 105°C). The volatile organic acids and alcohols were determined using HPLC. Determination of the DM content and subsequent correction were carried out using the following methods M0 – M4 (Figure. 1). The data set was divided into three DM-classes „15 - 20% DM“ (n=30), „20 - 25% DM“ (n=41) and „25 - 40% DM“ (n=25). The 5 methods (M0 - M4) were compared with each other using the nonparametric Kruskal-Wallis test. Individual pairwise comparisons were carried out using the Dunn's test. All statistical analyses were performed using R version 4.4.1.

M0. DMc/DMu =1 (without correction)

M1. According to Weißbach & Strubelt [2]

M2. According to Weißbach & Strubelt [2] with the addition of the volatility coefficient of FA (+ 0,9204 FA) [3]

M3. According to Huida et al. [3]

M4. According to Weißbach & Kuhla [1]

Results: The addition of formic acid influenced the DM content. This could be confirmed by significant difference between M1 and M2 within every DM-class ($p < 0.05$). If the FA content was neglected (M1), the DM was underestimated in the same way as without DM correction (M0). The extended method M2 simplified the calculation by using a formula without loss of accuracy (M3). There was no difference between M2 and M3 identified in any DM-class. M4 produced significant higher deviations at low DM contents (15 - 25% DM). At higher DM contents (25 - 40% DM) no difference between M4 and M2 or M3 were observed.

Conclusions: It is necessary to adapt the method for DM correction in order to adequately take into account the drying process and silage additives used. The results show that there is a need for further research.

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13.

Prevalence of gastrointestinal parasitism in cattle, sheep, and goats across sub-Saharan Africa: a meta-analysis

Prävalenz von gastrointestinalen Parasiten bei Rindern, Schafen und Ziegen in Afrika südlich der Sahara: eine Meta-Analyse

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Sub-Saharan Africa (SSA) is home to an estimated 1.1 billion heads of cattle, sheep, and goats (CSG), which contribute significantly to the food, income, and nutritional security in the region. Yet, their productive and reproductive performance has remained low compared to other regions worldwide. While inadequate nutrition of CSG is predominantly cited as the main limiting factor of ruminant production in SSA, it is well established that gastrointestinal parasites (GIPs) are a major issue affecting grazing animals [1]. However, there is no study on the extent of GIPs infestation in CSG within SSA. Therefore, using meta-analysis of proportions, we summarized the existing literature to estimate the prevalence of GIPs infestation in CSG across SSA and identified those parasites most rampant in the sub-region.

Methods: Sixty-one studies from 16 countries in SSA were identified via a thorough online search conducted between September and December 2023. A study was included into our meta-dataset if it (i) was conducted in SSA up to 10 years prior (i.e., 2013 – 2023) to the start of this study; (ii) was written in English; (iii) estimated the prevalence of one or more GIPs in ruminant livestock based on fecal sample analyses by the McMaster technique; (iv) focused on one or more of the three ruminant livestock species targeted (i.e., cattle, goats and sheep); and (iv) clearly reported the total number of animals from which fecal samples were collected and the number that tested positively for GIPs infestation. The data extracted were checked for plausibility and sorted for further analysis. All data were checked for normality using the Shapiro-Wilk test, then the weighted average proportion estimated with a 95% confidence interval using a random-effects model to account for the intra- and inter-study variances [2]. More so, we quantified the inter-study heterogeneity using Cochran's Q (χ^2), I^2, and tau-squared (τ^2) statistics. Heterogeneity was deemed significant when $p < 0.05$ in the Cochran Q test and $I^2 > 50\%$. Given that more than five studies were available for each ruminant species, a subgroup analysis was performed by species (i.e., cattle, goats, and sheep) to obtain within-subgroup summary proportions.

Results: At species level, the most frequently identified GIPs across all CSG were Fasciola spp. (n=51), Trichuris spp. (n=46), and Strongyle spp. (n=34). The pooled mean prevalence of GIPs in CSG was 63.3% (95% CI, 57.2 – 69.3). According to our sub-group analyses, the prevalence of GIPs infestation was 70.3% (95% CI, 60.8 – 79.0) in goats, 68.5 (95% CI, 58.6 – 77.6) in sheep, and 53.3 (95% CI, 49.5 – 60.5) in cattle, also suggesting there are differences in susceptibility between species. High heterogeneity was observed both across CSG and at the individual species level (i.e., I2 > 98%), indicating significant variation between the studies we considered. Also, no evidence of publication bias was found across all analyses.

Conclusions: This is the first study to provide an estimate of the prevalence of GIPs infestation across CSG in SSA. The combined mean prevalence of GIPs infestation across CSG in SSA over the last decade was high at 63.3% while at individual species level, it ranged from 53.3 – 70.3% on average. Given the central role of the gastrointestinal tract for the nutritional efficiency of CSG, further research is needed to elucidate how much gains in productive and reproductive performance can be achieved in these animals by reducing GIPs prevalence in the region.

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14.

Tissue-specific transcriptome signatures during mixed-parasite infections in chickens with different levels of growth performance

Gewebespezifische Transkriptom-Signaturen bei Mischparasiteninfektionen in Hühnern mit unterschiedlichen Wachstumsleistungen

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Co-infections with nematodes and protozoa occur frequently in free-range chickens and lead to complex local and systemic patho-physiological responses [1]. This study investigated tissue-specific transcriptomic signatures in chickens co-infected with Ascaridia galli and Heterakis gallinarum, later developing a *Histomonas meleagridis* infection. The study focused on 3 chicken strains with different growth rates (i.e. Lohmann Brown, LB; Lohmann Dual, LD; Ross-308, Ross) and on three tissues: jejunum, caecum, and liver, which are predilection sites for the parasite life cycle, and are central in host nutrient absorption, local and systemic immune responses, and metabolic regulation.

Methods: Male birds (n =12 per strain) were assigned to infection or control groups in a 3 × 2 factorial design. The infected groups (n = 18 birds) were inoculated orally with 0.2 mL NaCl with 500 infective eggs of *A. galli* and *H. gallinarum* at one week of age while the control group (n = 18 birds) was given a placebo (0.2 mL NaCl). Two weeks after inoculation, birds were killed to collect blood, jejunum, caecum, and liver tissue samples for plasma metabolite measurements and RNA sequencing, respectively. Transcriptomic data were generated, pre-processed and aligned to the current Gallus gallus reference genome (GRCg7b). The data was submitted to EMBL-EBI Annotare database with ArrayExpress accession number E-MTAB-14546. Analysis of differentially expressed genes (DEGs) was conducted using DESeq2considering a false discovery rate (FDR) < 0.05. Gene ontology (GO) enrichment analysis (Biological process, "BP") was performed using the topGO package in R.

Results: Infections led to significantly higher ascarid-specific antibodies ($P < 0.01$) and alpha-1-acid glycoprotein levels in plasma ($P < 0.01$). No significant infection effect nor interaction with strain was observed for average body weight gain (BWG) and feed intake (FI) with Ross having the highest BWG and FI ($P < 0.05$). Plasma concentrations of threonine, histidine, leucine, isoleucine were lower in infected birds ($P < 0.05$), but there were no differences in glucose, cholesterol, or NEFA levels ($P > 0.05$). Principal component analysis showed distinct clusters of infection vs. control for all three tissues, with the caecum showing the most extensive transcriptomic response (5,847 DEGs) due to infection (FDR <0.05). While 1,155 and 1,976 DEGs were found in liver and jejunum, respectively. A total of 4,094 DEGs was unique to the caecum. GO enrichment of all caeca DEGs showed a significant upregulation of both Th1 and TH_2 immune responses, wile mitochondrial ATP synthesis was suppressed ($P < 0.01$). In the jejunum, 760 unique DEGs were identified, with all significantly upregulated DEGs enriched in BP associated with muscle contraction, tissue repair, and intestinal motility, particularly in LB birds. Downregulation of BP associated to intestinal lipid absorption and proteolysis ($P < 0.01$) was also observed in the jejunum. In the liver, 266 DEGs were unique, with a marked prioritization of cytotoxic immune processes possibly aimed at parasite elimination, particularly in the Ross strain, over metabolic processes. Across all tissues, 199 genes were differentially expressed, with 41 inversely regulated between tissues. G-protein coupled receptor signalling and inflammatory response pathways were consistently upregulated, while processes related to fatty acid oxidation were downregulated across tissues.

Conclusions: These findings suggest that co-infections with *A. galli*,*H. gallinarum*, and *H. meleagridis* trigger tissue-specific immune and metabolic responses in chickens, with significant variation due to strain performance level. Immune pathways were predominantly upregulated, while metabolic processes were downregulated in all tissues. The transcriptomic signatures provide insights into how host adaptation occurs in response to multi-parasite exposure, which is a common challenge in outdoor poultry production systems.

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15.

Effects of tannin-rich extracts and plant materials from *Lythrum salicaria* and *Polygonum bistorta* on gene expression and immune regulation in the jejunum as well as microbial metabolites of weaned piglets

Auswirkungen von tanninreichen Extrakten oder deren Pflanzenmaterial von Lythrum salicaria beziehungsweise Polygonum bistorta auf die Genexpression und die Immunregulation im Jejunum sowie auf die mikrobiellen Metabolite im Darm von Absetzferkeln

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Tannin-rich plant extracts and materials from *Lythrum salicaria* and *Polygonum bistorta* can affect gut function, inflammatory processes and intestinal barrier function in piglets. These natural supplements can therefore support more sustainable and health-focused feeding strategies. It was the aim to investigate whether the addition of tannin-rich extracts or plant material of *Lythrum* or *Polygonum* can be utilized as a feed supplement to prevent weaning diarrhoea.

Methods: Weaned piglets (n = 80; 24 days old) were distributed to five groups. Each feeding group consisted of eight pens with two piglets each: control (CON), *Lythrum* extract (LSE), *Polygonum* extract (PBE), *Lythrum* plant (LSP), *Polygonum* plant (PBP) (supplementation of ~ 0.2 % tannins in treatment groups). After 12 d, tissue from jejunum was collected for gene expression analysis in the gut wall. The analysis was performed using Nucleo Spin RNA Plus (Macherey-Nage GmbH & Co. KG, Düren Germany). Digesta (ileum and colon) was analysed for short chain fatty acids as main microbial metabolites using a gas chromatograph. Software Rest was used to determine differences regarding gene expression between the groups ($p < 0.05$). One-way ANOVA followed by Tukey post-hoc test was performed for microbial metabolites.

Results: No difference was observed regarding the average daily gain, the feed intake or the faecal score between the groups ($P = 0.382$; $P = 0.109$; $P > 0.05$). Acetate was higher in ileum digesta in LSE compared to LSP ($P = 0.028$). In colon digesta, i-butyrate was higher in PBE than in LSP ($P = 0.023$). CON, LSE, PBE and PBP showed no difference in gene expression. LSP differed from the four other groups. IL-8, IL-17 and CLDN-2 was down regulated in LSP compared to CON ($P = 0.045$; $P = 0.003$; $P = 0.004$). LSP had a lower expression of TNFa, IL-6, IL-8 and IL-17 ($P = 0.038$; $P = 0.013$; $P = 0.021$; $P = 0.010$) compared to LSE. Moreover, LSP showed a downregulation in IL-17 and CLDN 2 ($P = 0.004$, $P = 0.021$). Compared with PBP, LSP was associated with a lower expression of MUC1 and IL-17 ($P = 0.044$; $P = 0.038$).

Conclusions: Results show that LSP supplementation might influence epihelial intestinal barrier functions and factors that regulate the immune function. Further research may confirm its potential as a sustainable alternative to conventional additives in animal nutrition.

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16.

How does feeding a purified diet alter gastrointestinal parameters of C57BL/6J mice?

Wie verändert die Fütterung einer „purified diet" gastrointestinale Parameter von C57BL/6J Mäusen?

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For feeding trials in laboratory rodents, either a standard diet or a specific control diet is used in the non-experimental groups. Since many experimental diets are purified, it could be relevant whether a purified or a standard diet is used as control. The aim of the present study was to compare such diets fed to mice in terms of gastrointestinal pH, short chain fatty acids (SCFA) and morphology.

Methods: Twenty-eight 8-week-old C57BL/6J mice were allocated to one of two diet groups fed either a pelleted standard diet for mice and rats (CON, n=14, 58% NfE, 25% crude protein, 6% crude fat, 5% crude fibre, all in dry matter; diet based on cereals and soy) or a pelleted purified control diet for rats and mice (PD, n=14, 57% NfE, 19% crude protein, 6% crude fat, 3% crude fibre, all in dry matter; diet based on purified starch and protein sources). Body weight (BW) was determined weekly. After 6 weeks on the respective diet, the mice were sacrificed (ethical approval CAM015/TWZ) and immediately dissected. Blood glucose was measured from cardiac blood, and gut sections and the liver were weighed. Of 7 mice per group, the pH of stomach, small intestinal, caecum and large intestinal content was measured (Mettler Toledo pH-meter). SCFA content in caecum and colon content was analysed by gas chromatography. Of the other 7 mice per group, the caecum and the large intestine were weighed in toto. The groups were compared by t-tests ($p=0.05$).

Results: From week 3 to week 5, the PD mice had a significantly lower BW than the CON mice ($p<0.001/<0.01/<0.05$, respectively). Blood glucose and relative liver weight (% BW) did not differ significantly between the groups ($p=0.89$). The relative caecum (means±SD: CON: 2.35±0.73 g; PD: 0.91±0.15 g; $p<0.001$) and colon (means±SD: CON: 1.68±0.55 g; PD: 0.82±0.20 g; $p<0.01$) weight was significantly lower in the PD group than in the CON group. The pH values of stomach, caecum and large intestinal content was significantly higher in the PD mice than the CON mice (for all three $p<0.0001$). In the caecum content, acetic (means±SD: CON: 48±6.3 mmol/L; PD: 34±5.8 mmol/L; $p<0.005$) and n-butyrate (means±SD: CON: 17±2.8 mmol/L; PD: 5.5±0.84 mmol/L; $p<0.0001$) contents were significantly lower in the PD group than the CON group. In the colon, these significant differences were also present and in addition, the level of propionate was significantly lower in the PD mice (means±SD: CON: 4.3±0.62 mmol/L; PD: 2.5±0.68 mmol/L; $p<0.001$).

Conclusions: The high caecum pH in combination with the significantly lower SCFA levels and the small size of this organ in the PD mice indicates a lower fermentative activity. A likely explanation for this is the assumed high digestibility of the purified diet made of single-nutrient ingredients (e.g. isolated starch / protein sources) in contrast to the cereal- and soy-based "natural" standard diet. The findings from this study highlight the different effects of diet types, i.e. standard/natural vs. purified, on the gastrointestinal physiology and metabolites of mice. Since the intermediary metabolism and the intestinal microbiome are likely to be affected as well, a relevant influence on the animal and the experimental data can be expected.

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17.

Effects of increasing proportions of rye in diets for broiler chickens after the 2nd week of life on caecal microbiota and bacterial fermentation products

Auswirkungen eines steigenden Roggenanteils im Futter von Masthühnern ab dem 14. Lebenstag auf die Mikrobiota des Caecums und bakterielle Fermentationsprodukte

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Life Cycle Assessment (LCA) methodology indicates that rye (Secale cereale) has lower environmental impacts compared to other cereal crops [1]. Therefore, rye could offer benefits in terms of climate change mitigation if it partially replaces traditional cereal grains in broiler diets. However, due to the higher presence of soluble non-starch polysaccharides, particularly arabinoxylans, its inclusion can be associated with reduced performance, sticky droppings, and changes in intestinal bacterial populations, especially in young broilers [2]. The gradual addition of rye to grower and finisher broiler diets (after the 2nd week of life) had no effect on excreta viscosity from day 14 to day 41, or on the viscosity of ileal digesta at day 42 [3]. The current study aimed to further evaluate whether this gradual addition of rye in broiler diets after the 2nd week of life affects caecal microbiota and bacterial fermentation products.

Methods: A total of 256 broilers (Ross 308) were randomly assigned to 32 pens on day seven and offered four different diets (eight replicates each) from day 14 to day 42. The control group was fed a conventional finisher diet based on wheat and soybean meal (Control). In two further groups, two pelleted supplementary feeds (SFI and SFII) were added to either crushed corn (SFI-Corn) or squashed rye (SFII-Rye). One mixture of 50% SFI-Corn and 50% SFII-Rye was given to the fourth group (Mixed). Weekly increases in cereal level (5%, 10%, 20%, and 30%) were made at the expense of the supplementary feeds (SFI and SFII). At day 42, the caecal contents of three birds per replicate (n = 24 per group) were obtained for microbiota (16S rRNA gene sequencing) and short-chain fatty acids (SCFA) analyses. Selected alpha diversity indices (Observed and Shannon index) were calculated using the R-package "phyloseq" (version 1.36.0). The microbiota composition was assessed for changes in relation to the factor diet by permutational multivariate analysis of variance (PERMANOVA) via the adonis function of the "vegan" package (version 2.5.7) using Bray-Curtis distance. Differentially abundant amplicon sequencing variants (ASVs) were identified with the help of the R-package "DESeq2" (version 1.32.0), which uses tests based on the negative binomial distribution.

Results: The diet had a significant effect on bacterial richness (Observed, $p = 0.0246$) and evenness (Shannon index, $p < 0.001$), as well as on composition ($p = 0.001$, $R^2 = 0.119$). Means of Observed and Shannon index were highest in SFI-Corn and lowest in SFII-Rye group. The microbiota composition of birds in the SFII-Rye group differed notably from Control and SFI-Corn, while the Mixed group displayed an intermediate position. Compared to the Control, several ASVs from the families Bifidobacteriaceae and Lactobacillaceae, as well as from butyrate-producing genera (Butyricicoccus, Anaerostipes, Ruminococcus, Erysipelatoclostridium and Subdoligranulum) were enriched in caeca of SFII-Rye birds. Total SCFA concentrations did not differ between groups ($p = 0.730$). However, when rye was included in the compound feed of the SFII-Rye and Mixed groups, the percentage of acetic acid in the total SCFA concentration decreased significantly from 81% (Control and SFI-Corn) to about 76% (SFII-Rye and Mixed; $p < 0.001$), while the percentage of n-butyric acid increased from 13% in the Control and SFI-Corn groups to 18% in SFII-Rye and Mixed groups ($p < 0.001$).

Conclusions: Overall, the inclusion of rye seems to alter the caecal microbiota and bacterial fermentation products, even when introduced in broiler diets starting from day 14. With the inclusion of rye, several bacterial taxa from butyrate-producing genera were enriched, leading to a higher percentage of n-butyric acid within the total SCFA concentration. Whether the inclusion of rye in broiler diets after the 2nd week of life has prebiotic potential and increases chickens' resilience remains to be evaluated.

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18.

Impact of *in vitro* faecal fermentation metabolites from mountain arnica (*Arnica montana*) on the growth of a porcine pathogen Escherichia coli

Der Einfluss von Berg-Arnika (Arnica montana) auf in vitro fäkale Metaboliten auf das Wachstum von pathogenen Escherichia coli

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Several medicinal plants are used in pig nutrition for their health benefits, particularly as natural alternatives to antibiotics, growth promoters, or for improving overall health [1]. One of the most important pathogens in pigs is enteropathogenic Escherichia coli, mostly affecting piglets after weaning (post-weaning diarrhoea), while sows can be a reservoir of this bacterium [2]. Mountain arnica (*Arnica montana*) is known for its beneficial properties in human medicine, however its effect on various aspects related to porcine health has not been tested yet [3]. Thus, the hypothesis of this study was that the metabolites produced during the *in vitro* fermentation of porcine faecal microbiota with dry mountain arnica inhibit the growth of pathogenic *E. coli*.

Methods: Dry mountain arnica at a concentration of 1 %, 5 % and 10 % was incubated anaerobically with pooled sow faeces. Negative control consisted of faecal incubation without arnica. After incubation, the fermentation slurry was centrifuged and sterile-filtered. The filtrate (sterile faecal water) was supplemented with the growth media and a pathogenic *E. coli* strain Abbotstown. The growth kinetics (OD_{600nm}) of *E. coli* was monitored during an overnight incubation. Exponential growth model was used to calculate growth rate, doubling time and lag time. The data were analysed using SPSS v.29.

Results: Sterile faecal water, obtained from fermentations at all concentrations of mountain arnica, significantly inhibited *E. coli* growth, increasing both its doubling time and lag phase compared to the control (*E. coli* growth in standard media) ($p \leq 0.05$). The strongest inhibitory effect on *E. coli* growth was observed at a 10 % concentration of mountain arnica.

Conclusions: Faecal fermentation metabolites of mountain arnica demonstrate potential to inhibit the growth of porcine enteropathogenic *E. coli*. Future studies should explore the antagonistic effects of mountain arnica against other porcine pathogens and investigate its underlying mechanisms of action.

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19.

The influence of dietary fiber on the gut bacterial quantity and activity in sows and newborn piglets

Der Einfluss von diätetischen Faserstoffen auf die bakterielle Gemeinschaft und Aktivität im Darm von Sauen und neugeborenen Ferkeln

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The relationship between sow and offspring is a key factor in microbial development. Modifying the sows‘ microbiota through nutritional interventions could potentially influence the piglets‘ microbial development and overall health [1]. Dietary fiber can modulate the gut microbiota composition and activity, producing numerous bioactive compounds in the intestine [2]. Therefore, it was hypothesized that a sow diet rich in high- or low-fermentable fibers with different particle sizes during gestation and lactation could differently affect bacterial colonization and activity in their newborn offspring.

Methods: Sows were fed isoenergetic and isonitrogenous diets with either 10% hay (HAY) or sugar beet pulp (SBP) of either fine (F) or coarse (C) particle size during gestation and lactation. Five sows were used in each of the HAY-F, HAY-C, and SBP-F groups, while six sows were used in the SBP-C group. Rectal contents were collected from the sows and their offspring (n=4/sow) one week after farrowing. Following DNA extraction, the fecal concentration of bacteria was measured using the qPCR method. In addition, rectal contents were assessed for SCFA (gas chromatography). Data were analyzed by Kruskal-Wallis with Mann-Whitney post-hoc tests, where applicable (SPSS v. 29). Significant differences were considered at $p \leq 0.05$.

Results: In sows, the concentration of bacteria and SCFA showed no difference. The concentrations of Clostridium cluster 1, enterobacteria and *E. coli*-Hafnia-Shigella were higher in the feces of piglets whose dams were fed SBP-C vs. HAY-C ($\log_{10}$ 8.50 vs. 9.23 gene copy/g, $p = 0.009$; $\log_{10}$ 7.48 vs. 8.05 gene copy/g $p = 0.003$, $\log_{10}$ 8.39 vs. 8.96 gene copy/g $p = 0.008$, respectively). The concentration of Streptococcussuis was higher in the feces of piglets whose dams were fed HAY-C vs. SBP-F ($\log_{10}$ 6.85 vs. 5.84 gene copy/g, $p = 0.050$) and SBP-C vs. SBP-F ($\log_{10}$ 6.82 vs. 5.84 gene copy/g, $p = 0.018$). The increased tendency of i-valerate and n-valerate concentrations were observed in piglets whose dams were fed SBP-F vs. HAY-C ($p = 0.071$, $p = 0.077$).

Conclusions: Dietary fiber type and particle size have no impact on the concentration of certain bacteria and SCFA in sow feces one week after farrowing. Fiber fermentability and particle size in sow diets can differentially alter the levels of certain bacterial populations but have minimal impact on microbial metabolites in the feces of one-week-old offspring.

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20.

Varying starch supply seems to affect phosphate transporters in the equine intestine

Auswirkungen einer unterschiedlichen Stärkezufuhr auf die Phosphattransporter im Pferdedarm

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Phosphate (P_i) movements along the gastrointestinal tract differ substantially among different species. In horses, functional data suggest a secretion in the small intestine and absorption occurring in the hindgut. In line with this assumption, NaPiIIb (sodium-phosphate-cotransporter type IIb) which is considered the most efficient transporter for intestinal P_i absorption was reported to be highly expressed in the equine colon, while PiT1 (phosphate transporter 1) was also found in the small intestine [1]. A positive precaecal digestibility of P was only measured when feeding compound feed but it is unclear whether inorganic P sources played a role or whether other mechanisms were responsible [2]. The present study aimed to describe the transporter expression pattern of P_i transporters in more detail. In addition, the effect of different amounts of concentrate was investigated. We assumed that the level of starch in the diet would have an influence on P_i transporter expression for two reasons: Firstly, altered microbial fermentation might induce an adaptation of P_i transporter expression as luminal P_i is not only a buffer and a nutrient for the microbiota but is also released from phytin-P by microbial enzymes. Secondly, variations in energy supply to the enterocytes might affect intracellular P_i homoeostasis and thus P_i transport across the plasma membrane.

Methods: For this purpose, 18 horses were divided into three feeding groups: While one group received only hay *ad libitum* (no-ST), two other groups were additionally fed 1 g or 2 g starch (ST)/kg body weight/day (ST1 or ST2), given as one meal of oat grains (474 g ST/kg dry matter; DM) in the morning. The lower dosage is recommended as a maximum intake per meal by the Society of Nutrition Physiology (GfE) to prevent equine gastric ulcer syndrome. Hay and oats contained 2.1 and 4.1 g P/kg DM. After at least 34 days, the animals were euthanised and tissue samples were taken from 12 distinct sites along the gastrointestinal axis. The expression of NaPiIIb was determined by quantitative RT-PCR. Overall, two-way ANOVA (feeding group, localisation) was performed. For selected localisations, the data were regrouped (ST vs no-ST; ≤ ST1 vs ST2) and analysed using Student‘s t-test or Mann-Whitney-test depending on the data distribution.

Results: Two-way ANOVA revealed a significant effect of the localisation. As expected, epression was markedly higher in the hindgut (right and left vetral colon, pelvic flexure, left and right dorsal and descending colon) than in the small intestine (duodenum, proximal, mid and distal jejunum and ileum; $p < 0.05$). Expression in the caecum differed neither from that detected the small intestine nor in the left dorsal colon. For evaluation of a diet effect, animals were regrouped as we assumed that in the small intestine, starch might have an effect even when fed at the lower level, while only higher amounts would impact the hindgut. In the proximal jejunum (PJ), NaPiIIb expression was higher in ST than in no-ST animals ($p < 0.05$). In the right dorsal colon (RDC), the expression was increased in ST2 in comparison to ≤ ST1 horses ($p < 0.01$).

Conclusions: It might be speculated that an increased abundance of luminal glucose in ST horses exerts an influence on NaPiIIb expression in the PJ. Such effects have been demonstrated in rats [3]. The observed increase in NaPiIIb RNA abundance in the RDC in ST2 animals could also be associated with the release of phytin-P from grains in the large intestine.

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21.

Optimizing the incubation solution for *ex vivo* investigations on intestinal tissues of chickens

Optimierung der Inkubationslösung für Ex-vivo-Untersuchungen an Darmgeweben von Hühnern

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The Ussing chamber technique is widely applied in different animal species, including cattle, sheep and pigs, where the incubation of gastrointestinal tissue can be sustained for hours. Chicken intestine is known to be significantly more delicate, resulting in difficult handling procedures and short viability within the Ussing chamber system [1]. The present study aimed to improve the tissue viability of chicken intestine *ex vivo* by optimizing the composition of the incubation solution.

Methods: Jejunum and cecum from a total of 18 broilers were used in the Ussing chamber under short-circuit conditions. A bilateral theophylline (8 mM) challenge was performed at 30, 60 or 90 min to check tissue viability. The following compositions of incubation solutions were tested for both tissues: (I) bilateral glucose (Glc) concentration (10 mM vs. 20 mM; N = 6), (II) bilateral Ca^{2+} concentration (1.5 mM vs. 3 mM; N = 6) and (III) serosal amino acid mix (present vs. absent; N = 6). Response variables included the baseline values of short circuit current (SC_{bl}) before the theophylline challenge and the maximum increase of short circuit current (Δ_{Imax}) within 10 min after the theophylline challenge. Data was analyzed using a two-way ANOVA with a post-hoc Student-Newman-Keul's test. Data are means ± SEM.

Results: (I) SC_{bl} in jejunum was higher at 20 mM vs. 10 mM Glc (24.0 ± 2 µA·cm-² vs. 12.7 ± 2 µA·cm-²) ($P < 0.001$) independent of the factor "time". (II) The jejunal SC_{bl} was influenced by an interaction effect ($P \leq 0.05$), reflected in a higher SC_{bl} at 3 mM vs. 1.5 mM Ca^{2+} at 30 min (20.3 ± 2.1 µA·cm-² vs. 4.5 ± 2.1 µA·cm-²) and 90 min (19.3 ± 2.1 µA·cm-² vs. 7.8 ± 2.1 µA·cm-²). Further, the ΔImax in cecum did not differ between 30 (55.0 ± 2.9 µA·cm-²) or 60 minutes (50.8 ± 2.9 µA·cm-²), but decreased after 90 min (41.8 ± 2.9 µA·cm-²) ($P \leq 0.05$) independent of the factor "concentration". (III) Serosal amino acids had no influence on any measured variable ($P > 0.05$).

Conclusions: These results suggest that increased concentrations of 20 mM Glc and 3 mM Ca^{2+} can improve the longevity of chicken jejunum and cecum *ex vivo*, supporting viability up to 90 min.

Acknowledgement: This study was supported by Evonik Operations GmbH.

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22.

Modification of endoplasmic reticulum stress-induced effects on expression of tight junction proteins and inflammatory and apoptosis-related genes by 1,25-dihydroxy-vitamin D_3 in 2D and 3D cultures of the porcine intestinal epithelial cell line IPEC-J2

Beeeinflussung endoplasmatischer Retikulum-Stress-induzierter Effekte auf die Expression von Schlussleistenproteinen und inflammatorischen und Apoptose-verwandten Genen durch 1,25-Dihydroxyvitamin D_3 in 2D und 3D-Kulturen der porzinen intestinalen Epithelzelllinie IPEC-J2

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The conventional two-dimensional (2D) culture of the porcine intestinal epithelial cell (IEC) line IPEC-J2 is an established *in vitro*-model of the porcine small intestine that is widely used in animal nutrition research to investigate the mode of action of nutrients and other bioactive substances in IEC. However, the disadvantage of the conventional 2D culture of IPEC-J2 cells is that IEC function is studied under unphysiological conditions with limited contact between adjacent cells and the lack of interaction between cells and the extracellular matrix (ECM), which limits the ability of transferring knowledge to the *in vivo*-situation. Thus, the aim of the present study was to establish a more convincing and meaningful three-dimensional (3D) culture of IPEC-J2 cells, which allows to study cell function in a more tissue-like environment, and to compare the effect of the endoplasmic reticulum (ER) stress inducer tunicamycin (TM) on ER stress indicators and the expression of tight junction proteins (TJP), inflammatory and apoptosis-related genes and the modulatory role of 1,25-dihydroxy-vitamin D3 (1,25D3) on these parameters in 2D and 3D cultures of IPEC-J2 cells.

Methods: For the 2D cell culture, IPEC-J2 cells were cultured in Dulbecco´s Modified Eagle Medium (DMEM) growth medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin and streptomycin. For the 3D cell culture, IPEC-J2 cells were plated in Matrigel-coated culture plates and incubated for 20-30 min to allow the cells to attach to the Matrigel. Afterwards, a mixture of Matrigel and growth medium without antibiotics (10:90, v/v) was added to the culture plates and spheroids were allowed to form over a period of 5 d during which Matrigel-medium mixture was replaced every 2 d. Both, 2D IPEC-J2 cells and differentiated 3D IPEC-J2 spheroids were treated either without (0.1% DMSO alone) or with TM or with TM and 1,25D3 for 24 h. Expression of ER stress indicators, TJP, inflammatory and apoptosis-related genes was determined by qPCR, immunoblotting and/or immunocytochemistry. Statistical analysis was performed using the Minitab statistical software (Rel. 13.0, State College, PA, USA). Data were subjected to one-way ANOVA. For statistically significant F values, individual means of the treatment groups were compared by Fisher's multiple range test. Effects were considered significant if $P<0.05$.

Results: A published protocol for 3D culture of Caco-2 cells [1] was successfully adopted to IPEC-J2 cells which was evident from fully differentiated 3D IPEC-J2 spheroids showing the characteristic spherical architecture with a single layer of IPEC-J2 cells surrounding a central lumen. Treatment of 2D IPEC-J2 cells and 3D IPEC-J2 spheroids with TM for 24 h markedly increased mRNA and/or protein levels of the ER stress target genes, heat shock protein family A (Hsp70) member 5 (HSPA5) and DNA damage inducible transcript 3 (DDIT3) ($P<0.05$), whereas co-treatment with TM and 1,25D3 did not mitigate TM-induced ER stress in IPEC-J2 cells in the 2D and the 3D cell culture. In contrast, TM-induced expression of pro-inflammatory (interleukin-6 (IL6), IL8) and pro-apoptotic genes (BCL2 associated X, apoptosis regulator (BAX), caspase 3 (CASP3), CASP8) and genes encoding TJP (TJP1, claudin 1 (CLDN1), CLDN3, occludin (OCLN), cadherin 1 (CDH1), junctional adhesion molecule 1 (JAM1)) was reduced by co-treatment with TM and $1,25D_3$ in 3D IPEC-J2 spheroids ($P<0.05$), but not in the 2D cell culture.

Conclusions: While the ER stress inducer TM causes a pronounced induction of the ER stress marker genes HSPA5 and DDIT3 in both IPEC-J2 cell culture models, the effect of $1,25D_3$ in the IPEC-J2 cell culture is dependent on the culture model applied. The observations in the more meaningful 3D IPEC-J2 cell model indicate that $1,25D_3$ partially protects from TM-induced activation of pro-inflammatory and pro-apoptotic signaling.

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23.

mRNA levels of trace amine-associated receptors (TAAR) throughout the bovine gastrointestinal tract – novel sensors for feeding changes?

mRNA-Expressionslevel von TAARs entlang des bovinen Gastrointestinaltrakts – bislang unbekannte Sensoren für Fütterungsumstellungen?

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Dairy cows require high energy feeding to cover their energy needs for milk production. Although the forestomachs are originally designed to ferment complex carbohydrates to short chain fatty acids, the bovine gastrointestinal epithelium can adapt to absorb simple sugars to some extent (1, 2). This regulation is not only crucial to cover the animal's energy demand but also to maintain a stable milieu in the gastrointestinal lumen. Hence, we hypothesized that there are sensors in these epithelia that mediate an adaptation to increased portions of concentrate in the diet. Trace amine-associated receptors (TAAR) may have a role in this sensing, since they bind biogenic amines, which are increasingly produced by the ruminal microbiota after increasing the concentrate portion in the ration (3) and might thus indicate the need for adaptation.

Methods: The animal experiment was part of the DFG funded project „Host-Microbiome Interaction: Implications for the cellular and global energy metabolism in the dairy cow (WiMiQ)" (202989534). 20 primiparous Holstein Frisian cows were fed with a ration either meeting or exceeding their energy needs (N=10 each). After six weeks, the animals were slaughtered and epithelial samples from reticulum, ventral sac of the rumen, omasum, and jejunum were collected. mRNA was extracted from these samples and the gene expression of TAAR1, TAAR4 and TAAR9 was measured using RT-qPCR. Peptidylprolyl isomerase A, beta-actin, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta, ribosomal protein like 4 and 32 served as reference genes. The expression rates determined by the ddCt method were compared using Student's t-test and assuming a statistically significant difference if $p<0.05$.

Results: All three target genes were detected in all of the tissues. However, the pattern of mRNA abundance of the different TAARs differed qualitatively between the locations. In particular, TAAR1 was hardly present in rumen and omasum, but abundant in reticulum and jejunum epithelium. TAAR4 and TAAR9 mRNA appeared to be more abundant in all tissues but again more reliably expressed in reticulum and jejunum epithelium. There were no statistically significant differences between the two feeding groups for any of the genes.

Conclusions: We could show that TAAR1, 4 and 9 are present in epithelia along the bovine forestomachs and small intestine, albeit at different abundances. This may suggest different sensitivities in the reticulum and jejunum compared to rumen and omasum. Feeding different energy levels did not influence the mRNA expression levels. However, the sensing of energy content and regulation of transepithelial transport mechanisms might take place on protein level or via second messenger systems, which remains to be elucidated in future studies.

Acknowledgement: The animal experiment was part of the DFG funded project „Host-Microbiome Interaction: Implications for the cellular and global energy metabolism in the dairy cow (WiMiQ)" (202989534).

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24.

Amino acid digestibility and digestive enzyme activities in pigs fed diets containing different pea varieties

Aminosäurenverdaulichkeit und Aktivität von Verdauungsenzymen von Schweinen bei Fütterung von Rationen mit unterschiedlichen Erbsensorten

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The energy and protein values of peas (Pisum sativum) are key criteria for their incorporation into pig diets and accurate diet formulation includes nutrient digestibility data. Peas contain variable amounts of antinutritional factors that may limit the digestibility of nutrients, e.g. by affecting the activity of digestive enzymes [1]. The effect of different pea varieties on the activity of digestive enzymes in the small intestine of growing pigs has not been studied yet. We hypothesise that the activity of digestive enzymes and the precaecal digestibility of amino acids may be affected by the characteristics of different pea varieties.

Methods: Four diets based on wheat (20%), barley (15%), soybean meal (10%), and rapeseed meal (10%) were formulated with 40% of either a spring pea (diet1), one of two winter peas (diet2 and diet3), or a spring pea with higher tannin content (diet4). Titanium dioxide was included as an indigestible marker. Eight barrows (25.7 ± 0.9 kg initial BW) were fitted with a T-cannula at the distal ileum and assigned to the four dietary treatments in a 4×4 double Latin-square design. Daily feed allowance was 4% of the mean BW. The experiment included 4 experimental periods of 11 days each with 7 days of adaption to the diet, followed by 2 days of faeces collection and 2 days of ileal digesta collection. Additional samples of ileal digesta and faeces were collected on the last day of each period to determine enzyme activities of carboxypeptidase A and B [2], trypsin, chymotrypsin, and amylase. Data were analysed with a linear mixed-effects model using the lmer package in R. The model included the dietary treatment as fixed effect and the animal and the experimental period as random effects. Pairwise post-hoc comparisons were computed using the emmeans package. Correlations were performed using Pearson correlation coefficient and P-values were controlled for multiple testing by the Benjamini-Hochberg procedure.

Results: There were no significant differences among the diets in the precaecal digestibility of DM, CP, P, Ca, and GE. The precaecal digestibility of all amino acids except for Gly was affected by the dietary treatment ($P < 0.05$). The precaecal digestibility of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val, Asx, Ser, and Tyr was higher for diet1 than for diet2 and diet3 ($P < 0.05$) with differences between 2.8%-units (Met) and 5.2%-units (Ser). In ileal digesta, the activity of trypsin, chymotrypsin, and carboxypeptidase A was affected by the diet ($P < 0.05$), but not the activity of amylase and carboxypeptidase B. Trypsin and carboxypeptidase A activity was higher for diet2 and diet3 than diet4 ($P < 0.05$). For diet1, the carboxypeptidase A activity was lower than for diet3 ($P < 0.05$). Diet4 decreased chymotrypsin activity compared to diet3 ($P < 0.05$). Negative correlations were found between the precaecal digestible concentration of all analysed indispensable amino acids and the activity of trypsin, chymotrypsin, and carboxypeptidase A ($P < 0.05$).

Conclusions: The results suggest that higher activity of proteolytic digestive enzymes in the distal ileum cannot be associated with greater precaecal amino acid digestibility of pea-rich diets. A lower protein digestibility of the peas might have caused higher enzyme secretion by the animal. The lower precaecal amino acid digestibility of diet2 and diet3 may be related to a higher trypsin inhibitor content found in winter pea varieties [3].

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25.

Particle size distribution of complete feed for laying hens – Differences between organically and conventionally produced feed

Partikelgrößenverteilung von Alleinfuttermitteln für Legehennen - Unterschiede zwischen ökologischer und konventioneller Erzeugung

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Appropriate particle size distribution in diets for laying hens ensures feed intake and nutrient supply in line with requirements and thus plays a decisive role in maintaining health and performance of the animals [1]. The current recommendations for grinding and particle size distribution used in practice are derivded from conventional diets for laying hens and the feed compounds frequently used therein. Considering the selection and grinding of different feeds for organic diets, it can be assumed that the particle size distribution may differ to conventional diets. An unbalanced particle size distribution can cause behavioural problems like picking behaviour, resulting in changes of nutrient intake and physiological pathways. The aim of the present study was to identify possible differences in the particle size distribution using diets of conventional and organic origin. Furthermore, selected nutrients of the whole diet and specified particle size proportion were compared. It was hypothesized that the particle size distribution and the nutrient composition of whole diet and specified particle size proportion differs between diets of conventional and organic origin.

Methods: The study was performed with 38 mash diets (phase I: 20-50 week of life; samples from the standard feed industry). Twenty diets were of organic and 18 of conventional origin. The diets were classified according to information regarding e.g., milling-technic and used feed compounds as well as analyzed nutrient composition (crude nutrients, starch, sugar, calcium, phosphorus, methionine and cysteine). Dry sivieng analyses were performed (1) using 9 sieves (0.2 to 3.15 mm; n=38) or (2) two sieves (0.5 mm and 2.0 mm; HAVER EML 200 Premium Remote®; Haver & Boecker; amplitude of 2.00 mm for 10 min; n=12). Crude ash (CA; No. 8.1), crude protein (CP; No. 4.1.2) and phosphorus (No; 10.6.1 [2]) were analyzed for each of the three samples per diet that derived from method (2). The nutrient content of the particle size proportions was calculated by multiplying the content of e.g. CA with the respective result of particle size distribution. Data were evaluated by statistic program R®, using a generalized (GLM) or linear model (LM) with ANOVA (F-test). A post-hoc test with Bonferroni correction was performed, with the P value set at < 0.05.

Results: The results for particle size distribution of method (1) showed differences between organic and conventional diets for 0.8 mm, 1.0 mm and 3.15 mm ($p < 0.05$) with higher values in organic diets for 3.15 mm (5.91 vs. 2.31 %) but lower values for 0.8 and 1.00 mm. For method (2) no differences were found for distribution to < 0.5 mm, 0.5 to 2.0 mm and > 2.0 mm. Organic diets showed higher content of crude fiber (57.3 vs 49.3 g in 88% DM) and ether extract (59.2 vs 39.2 g in 88 % DM) but lower values for starch (345 vs 377 g in 88 % DM). Comparing the content of nutrients in the specified particle size proportion of method (2) differences appeared in > 2.0 mm for CP with higher values for organic diets (18.00% vs 13.27%; $p < 0.05$) but not for CA (7.30% vs 8.28%) and phosphorus (0.38 % vs 0.29 %). The supply of CA, CP and phosphorus derived from particles of 0.5 mm, 0.5 to 2.0 mm and > 2.0 mm did not differ.

Conclusions: As hypothesized, some differences were found for particle size distribution between organic and conventional diets, with larger particles found in organic samples. Even though the content of CP in organic diets was higher in particles >2 mm, no further impact could be analyzed for the supply of CA, CP and phosphorus. Further studies should continue to analyze nutrients within specified particle size proportion, with respect to animal species. Thereby, new data should be implemented with the particle size distribution method currently under development at the VDLUFA.

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26.

In vitro gas production dynamics: Exploring mathematical models across feeds

Dynamik der in vitro-Gasproduktion: Untersuchung mathematischer Modelle für verschiedene Futtermittel

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In vitro gas production (GP) is a standard measure used in ruminant feed evaluation; however, the complexities involved in GP dynamics necessitate robust mathematical modelling for accurate interpretation. It is hypothesized that distinct feed types exhibit specific GP characteristics that particular models can effectively capture [1]. Therefore, efforts have been made to explore various nonlinear models and methods to explain GP dynamics across different concentrate feed types, addressing the question: How can efficient and versatile mathematical models accurately representing GP profiles be identified? Thus, this study aimed to evaluate a broad set of systematically chosen nonlinear mathematical models for their ability to fit *in vitro* GP data across different feed types and to utilize machine learning methods to identify the best-performing model or group of models that can efficiently describe GP curves across feeds.

Methods: A comprehensive dataset of 849 syringes with GP recorded over time in the Hohenheim gas test at our department was utilised. The studied concentrate feeds included cereal and leguminous grains, oilseed meals and other protein-rich feeds, and compound feeds. We initially screened 63 candidate models that we considered could effectively describe the GP dynamics based on their curve shapes. Through a rigorous evaluation process, we narrowed this selection down to 21 models that exhibited unique mathematical structures and provided a good fit for the entire dataset of different feeds. The Bayesian Information Criterion (BIC) was used for model evaluation and selection, effectively balancing simplicity and accuracy. After fitting the 21 models, we employed the machine learning decision tree method [2] to identify the top-performing model or group of models that could efficiently describe GP dynamics. In this way, we used the models and feed categories as inputs, while each model‘s BIC served as the output. We calculated Relative Performance Improvement (RPI), which measures how much top-performing models outperform lower-performing models relative to the average BIC within each feed category.

Results: The results showed that a group of three models—Burr XII (4 parameters), Inverse Paralogistic (3 parameters), and Loglogistic (3 parameters)—consistently emerged as top performers, demonstrating high generalizability and predictive ability across feeds. Analysis indicated that model type had a greater impact on GP predictions than feed type, contributing 65.2% compared to the 34.8% contribution from feed types. Calculated RPI values across the examined feed categories showed a noteworthy improvement ranging from 34% to 94% when using the best-performing models compared to the lower-performing ones. In terms of estimated parameters, such as maximum GP, time of inflection point, and half-life, the differences among the top three models were relatively minor within feed type. This suggests a degree of generalizability that allows any of these models to estimate aspects of GP dynamics for various concentrate feeds reliably.

Conclusions: A comprehensive analysis of models for fitting *in vitro* GP data across feeds, empowered by machine learning, identified three superior models: Burr XII, Inverse Paralogistic, and Loglogistic. These models demonstrated high accuracy, consistency, and generalizability, effectively capturing the diverse dynamics of GP across different feed types. The findings emphasise the importance of model selection in optimising predictive accuracy, as the choice of model significantly influenced GP predictions. This research provides a basis for future explorations into how model parameters relate to feed characteristics and their consequences for *in vivo* digestibility estimation.

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27.

Systematic comparison of gas production in batch culture incubations with rumen fluid or faeces as inoculum

Systematischer Vergleich von Gasproduktion in Batch Culture Inkubationen mit Inokulum aus Pansensaft oder Kot

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For rumen *in vitro* studies like the Hohenheim Gas Test (HGT) using rumen fluid as inoculum requires the use of cannulated ruminants. For decades, researchers have explored alternative inoculant sources, particularly faecal inoculum. In this study, we systematically compared gas production from rumen fluid (RF) and faecal inoculum (FI) across different studies, aiming to evaluate whether FI could potentially replace RF as an inoculum regarding gas production from *in vitro* batch cultures, and to identify the factors that influence the difference of gas production between RF and FI.

Methods: Out of the 11 studies identified through Web of Science and Google Scholar, 8 studies containing 589 observations on gas production from RF and FI were selected for inclusion into our metadata if they: (i) were published in English; (ii) collected RF and FI from the same animal; (iii) Incubated the same feed substrate with both RF and FI; and (iv) reported gas production volumes and the corresponding feed substrate information. Gas production volumes at 8, 24, 48, and 72 h were extracted directly from tables or from graphs using WebPlotDigitizer (Version 5.1), and standardized to mL per 200 mg of incubated dry matter. To assess the adequacy (i.e., accuracy and precision) of using FI as a substitute for RF in predicting gas production, the mean bias error (MBE), relative prediction error (RPE), and concordance correlation coefficient (CCC) were calculated. The adequacy assessment was conducted by comparing gas production in: (i) RF vs. FI across all incubation times (8, 24, 48, 72 h); (ii) RF at 24 h vs. FI at 48 h; (iii) RF at 24 h vs. FI at 72 h. Next, a multivariate regression model was fitted to relate the incubation parameters extracted from the different studies to the observed differences in gas production between RF and FI across incubation times. The donor animal type, donor animal diet, inoculum sampling method, inoculum mixing method, culture medium type, RF dilution rate, FI concentration (assuming dry matter content in faeces of 15% for cattle and 35% for sheep), incubated substrate type, incubated substrate mass, gas measurement method, and incubation time were included as potential explanatory variables into the initial multiple regression model. These variables were iteratively eliminated by removing that with the highest ($P>0.1$) P-value for each iteration until all variable P-values suggested at least a tendency ($P<0.1$) for significance ($P<0.05$) [1].

Results: The lowest MBE was observed when comparing RF at 24 h vs. FI at 72 h (4.70 mL/200 mg DM), followed by the comparison of RF at 24 h vs. FI at 48 h and across all incubation times (9.65 and 13.73 mL/200 mg DM, respectively). Similarly, the RPE was smallest for RF at 24 h vs. FI at 72 h (23.97%), followed by RF at 24 h vs. FI at 48 h (29.49%), and was largest across all incubation times (34.04%). The CCC showed the highest agreement for RF at 24 h vs. FI at 72 h (0.80), followed by RF at 24 h vs. FI at 48 h (0.72). The lowest CCC (0.72) was observed when comparing RF and FI across all time points. Among all considered explanatory variables, FI concentration, incubated substrate type, and incubation time were those responsible for the observed difference in gas production between RF and FI ($P<0.001$).

Conclusions: The gas production from FI at 72 hours demonstrated a better prediction of RF gas production at 24 h compared to FI gas production at 48 and 24 hours, as evidenced by the lowest MBE and RPE and the highest CCC. To improve the accuracy of predicting RF gas production at 24 h, more consideration should be given to the FI concentration, as a higher FI concentration decreases the difference between RF and FI gas production. Additionally, the microbial communities in RF and FI might exhibit different fermentation capacities depending on the substrate. Future studies should focus on the gas production kinetics and fermentation properties specific to individual feed types incubating RF and FI.

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28.

Estimation of dry matter intake of dairy cows using test milk MIR spectra

Schätzung der Trockenmasseaufnahme von Milchkühen unter Nutzung von MIR Spektren der Milch

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Dry matter intake (DMI) should be known as accurately as possible when formulating rations for dairy cows to determine the energy and nutrient concentrations needed to meet requirements, but also to avoid oversupply. In Germany, DMI is usually predicted using the equations developed by Gruber et al. [1], which include body weight (BW), milk yield (MY), parity (LN), days in milk (DIM) and breed, as well as feed-related parameters. However, DMI can additionally be influenced by factors that are not included in the equations. It is well known that energy balance affects milk fat composition [2]. Milk composition can be measured using mid-infrared (MIR) spectroscopy. The aim of the present study was to investigate whether milk fat composition or MIR spectral data can be used to improve the estimation of DMI in dairy cows.

Methods: Data on DMI, MY, BW, LN and DIM were collected from 23 Holstein-Friesian dairy cows for 10 weeks. All cows were fed the same total mixed ration (TMR) *ad libitum*. Milk samples were collected weekly and were analysed for MIR spectra using the MilkoScanTM 7 RM (Foss Analytics, Hillerød, Denmark), with milk constituents derived from the spectra. Daily the DMI was assessed (RIC2discover, Hokofarm Group) and MY was recorded, for both variables the average was calculated for the week before milk sampling. BW was measured biweekly. Five cows were in 1st lactation, six in 2nd, and twelve in 3rd or higher lactation. At enrolment the MY was 37.5 kg and the cows were in different lactation stages: ≤ 50 days in milk (DIM): n = 6; 51 – 100 DIM: n = 6; 101 – 200 DIM: n = 6; 201 – 300 DIM: n = 5. Individual DMI was estimated on a weekly basis using Gruber et al. equation 5 (G) [1]. Two multivariate regression models (MVR) were designed, one with the same predictors as in the Gruber model (MVR1) and one extended with de novo fatty acid (≤ 14 carbon atoms) proportions in milk fat (MVR2). To investigate whether the MIR spectra of the milk can improve the estimation of DMI, a partial least squares (PLS) model was used by extending MVR1 to include the MIR spectra of milk. To validate the models, cows were randomly split into a training set (n = 17) and a test set (n = 6), and model performance was assessed using R^2, root mean square error of calibration (RMSEC), and root mean square error of prediction (RMSEP).

Results: Ranking the models by R^2 values for training (MVR1, MVR2 and PLS) and prediction (G) on the whole dataset and lactation resulted in the highest value for MVR2 (0.77), followed by PLS (0.72), MVR1 (0.59) and G (0.47). This ranking is confirmed in reverse order by the corresponding RMSE values indicated in kg: RMSEC values were 1.61 for MVR2, 1.78 for PLS, 2.12 for MVR1 and RMSEP value for G was 2.49. These results were confirmed for the entire lactation by calibrating these models on the training set and a subsequent application on the test set. For the lactation stages model MVR1 showed the lowest RMSEP (2.81) until day 50. The highest value was observed for G at 4.57. MVR2 (3.78) and PLS (3.02) showed values in between. In the following lactation stages RMSEP was always lowest for MVR2 (51-100: 1.26, 101-200: 1.73, 201-400: 1.53). From DIM 51 to 100, MVR1 and PLS had lower RMSEP values of 1.36 and 1.84 than G (2.71). In the last two stages of lactation G showed lower RMSEP values of 2.34 and 1.78 while MVR1 and PLS had fluctuating and higher values (2.11 – 5.69).

Conclusions: These preliminary results should be interpreted with great caution as all models except G were calibrated on data collected under the same conditions as the very small test set. Nevertheless, the results suggest that milk constituents or milk MIR spectra can improve the estimation of DMI, especially in early lactation. This may be complementary to G, which had shown poorer adaption in early lactation and an accurate estimation from day 101 of lactation. Further data, collected under different conditions, are necessary to strengthen the robustness of the models.

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29.

Influence of partial replacement of crude protein from extracted rape seed meal by non-protein nitrogen from feed grade urea at varying space allowance on performance of fattening Fleckvieh bulls

Einfluss des teilweisen Ersatzes von Rohprotein aus Rapsextraktionsschrot durch Nichtproteinstickstoff aus Futterharnstoff bei variierendem Platzangebot auf die Mastleistung von Fleckviehbullen

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Ruminants can use nonprotein nitrogen from sources like urea by converting it via their microflora to amino acids. In a recent study [1] inclusion of 0.83 to 0.90 % of DM of feed grade urea in diets for fattening bulls decreased DM intake and tended to decrease growth rate. For this reason, the present study was conducted to evaluate, whether those effects would also appear at a lower rate of urea inclusion in diets for fattening bulls. A two-factorial arrangement was used to additionally examine the effects of space allowance on performance of fattening bulls.

Methods: 60 Fleckvieh bulls (age: 181±10 d, body weight (BW): 228±17 kg) were allocated by body weight, feed intake, and age to the feeding groups "control" and "urea". The bulls in the control group received a total mixed ration (TMR) based on maize silage, corn silage and concentrates including extracted rape seed meal (RSM) as the main protein sourcefor *ad libitum* intake. In the diets of group urea, feed grade urea (0.69 % of DM) and dried beet pulp was included at the expense of RSM to provide isoenergetic and isonitrogenous diets for both groups. A three-phased feeing regimen was used to adapt dietary crude protein concentration to the decreasing requirements over the fattening period. Within each feeding group, three subgroups (n8, n10, and n12) were built to evaluate the effect of space allowance on performance of bulls. Bulls of each subgroup were kept in two pens with a ground area of 37.5 m2 each. The stocking rate was 8, 10, or 12 animals per pen corresponding to a space allowance of 4.7, 3.7, and 3.1 m2 per bull. Individual feed intake was automatically recorded daily while BW was recorded every four weeks. The bulls were slaughtered at a mean age of 497 d. Data was evaluated by a two-factorial model with post hoc SNK comparison using SAS. Level of significance was set to $p<0.05$. Two animals were removed from the trial due to illness and substituted by replacement bulls. These replacement bulls were excluded from statistical analysis.

Results: There was no effect of diet formulation on DM, energy, and nutrient intake, except of P intake which was 40.2 and 31.8 g/d ($p<0.05$) in groups control and urea, respectively. Body weight at the end of the experiment and daily gains were 744 kg and 1658 g/d with no differences between feeding groups. There was no effect of diet formulation on carcass and fat classification, dressing percentage, and slaughter weight. Share force of meat (m. longissimus dorsi), drip losses, cooking losses, and intramuscular fat content were not influenced by diet formulation.DM intake was numerically lower ($p=0.138$) in subgroup n12 (9.3 kg/day) than in the other subgroups (both 9.8 kg/day) and consequently energy and nutrient intake was also numerically lower. End weight and daily gain did not differ between the space allowance groups. In the starter phase (day 1 to 105), however, daily gain numerically increased ($p=0.160$) with increasing space allowance (1853, 1893, and 1976 g/day for subgroups n12, n10 and n8) and the same tendencies were observed in the grower phase (1748, 1783, and 1867 g/d for subgroups n12, n10 and n8; $p=0.384$). In the finisher phase growth rate was not related to space allowance (1359, 1430, and 1264 g/d for subgroups n12, n10 and n8; $p=0.164$). Slaughter performance was not influenced by space allowance.

Conclusions: Partial replacement of crude protein from extracted rapeseed meal by non-protein nitrogen from0.69 % of DM feed grade urea has no impact on feed intake and growth performance of fattening bulls. Increasing space allowance from 3.1 m2 to 4.7 m2 per animals appears to have some negative impact on growth in young animals, but effects are less pronounced in the finisher phase.

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30.

Suitability of the Daisy Incubator II for estimating the organic matter digestibility of feedstuffs

Untersuchungen zur Eignung des Daisy-Inkubators II für die Schätzung der Verdaulichkeit der organischen Masse von Futtermitteln

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The study aimed to compare different methods for determining *in vitro* degradability (IVD) using the Daisy Incubator II (ANKOM Technology): "ELOS" (VDLUFA) a well-established method for estimating the enzyme-soluble organic matter (ELOS), "DAISY-ND (Neutral Detergent)" a method provided by ANKOM Technology, and "DAISY-T&T", a modification of the original Tilley and Terry method[2] to be conducted using the Daisy Incubator II (1999)[3]. In addition, data obtained for roughage and foliage mixes were compared to results from a feeding trial using sheep.

Methods: In experiment 1, we used maize silage (MS), grass silage (GS), hay and straw. Experiment 2 included diets that had already been used in a feeding trial to assess *in vivo* digestibility: 100 % hay, 64 % hay & 36 % Populus tremula (TREM), 64 % hay & 36 % Populus nigra (NIG), and 61 % hay & 39 % *Salix viminalis*(SAL). All feedstuffs were dried in a forced air oven (60 °C, until constant weight), ground to 1 mm and weighed into F57 filter bags. ELOS was conducted using pepsin and cellulase (VDLUFA, 6.6.1). For DAISY-ND, 0.25 g of substrate were incubated in 1596 ml buffer solution and 400 ml filtered rumen liquid for 24 h before boiling for 1 h in ND solution. For DAISY-T&T, 0.5 g of substrate were incubated in the same solution for 48 h and after the addition of 16000 FIP-U pepsin and 40 ml 6 N HCl the incubation was extended for another 24 h. IVD of OM was determined from the difference in weight before and after incubation corrected for crude ash content. All experiments were done using triplicates and carried out at least three times (N = 3 for straw, N = 7 for MS, GS and hay, N = 3 for the roughage and foliage diets). Digestibility of organic matter (OMD) was estimated from the IVD using the respective calculations for the roughages. A mixed model was applied to reveal differences between the methods dependent on the different feedstuffs and the impact of variations within the method. Linear regression analysis was conducted to determine the correlation between the contents of ADF and NDF in the different feedstuffs and the degree of IVD for the different methods.

Results: Except for ELOS and DAISY-T&T for MS and DAISY-ND and DAISY-T&T for straw, the IVDs determined with the different methods differed significantly ($p < 0.05$). IVD amounted to 76.6 ± 0.81% (ELOS), 87.8 ± 2.00% (DAISY-ND), and 77.6 ± 2.90% (DAISY-T&T) for MS, 77.9 ± 1.00%, 92.7 ± 1.02%, and 82.7 ± 2.17% for GS, 51.9 ± 1.23%, 76.5 ± 4.12%, and 62.4 ± 4.24% for hay, and 38.0 ± 1.97%, 54.8 ± 2.64%, and 45.0 ± 1.91% for straw. In general, ADF content and IVD were linearly correlated (R^2: 0.97, 0.91 and 0.94. However, the calculated slopes differed significantly between ELOS and the two methods containing rumen fluid ($p < 0.05$). The correlation between NDF and IVD was closer for ELOS (R^2: 0.92) than for DAISY-ND (R^2: 0.69) and DAISY-T&T (R^2: 0.77). In experiment 2, OMD calculated from ELOS, DAISY-ND and DAISY-T&T of hay amounted to 69.0 ± 0.27%, 76.4 ± 1.35%, 66.5 ± 1.28% compared to 70.4% determined *in vivo*, OMD of TREM to 69.4 ± 0.41%, 82.7 ± 2.01%, 61.6 ± 2.93% and 62.2 % *in vivo*, OMD of NIG to 70.5 ± 0.30%, 75.9 ± 0.90%, 66.5 ± 1.11% and 68.5% *in vivo*, and OMD of SAL to 70.8 ± 0.33%, 74.3 ± 2.00%, 66.4 ± 1.95% and 65.0% *in vivo*.

Conclusions: As ELOS relies on a standard amount of synthetic cellulase, this method shows the smallest variations between trials and a close correlation of IVD with the contents of ADF and NDF. But except for MS, higher IVD was determined for all feedstuffs when methods including rumen fluids were used indicating that a mix of microbial enzymes is needed to mimic digestion of feedstuffs rich in complex carbohydrates. DAISY-T&T seems to be a robust method suitable for the evaluation of OMD of different feedstuffs: The results obtained for MS were comparable to those generated using ELOS, and also those for the roughage & foliage mixes compared best to the *in vivo* data. DAISY-ND seems to overestimate OMD.

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31.

Effect of supplementing tree leaves from poplar and willow on the digestibility of hay- fed sheep

Einfluss einer Zulage von Pappel- und Weidenblättern auf die Verdaulichkeit von Heu-gefütterten Schafen

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Silvopastoral agroforestry systems, a combination of woody plants, pasture and livestock farming, enable mutual benefits to the environment and the animals living in it [1]. Besides grass, leaves from trees and hedges may provide an additional feed source to livestock. Poplar and willow are deep rooting trees, containing substantial amounts of crude protein and minerals such as Se [2]. On the other hand, topsoil and thus pasture grass are Se deficient in many areas in Germany [2], calling for the supplementation of minerals to livestock. Here we aimed to assess the nutritional value of poplar and willow leaves regarding the organic matter (OM), crude protein (CP), neutral detergent fiber (aNDFom), and energy digestibility, as well as the Se balance in sheep.

Methods: Four Coburger Fuchs wethers (~1 year, 50 kg body weight) were fed a hay-based ration supplemented either or not with 39-40% dried leaves from black poplar (*P. nigra* (NIG)), aspen (*P. tremula* (TREM)), or willow trees (*Salix viminalis* (SAL)) for three weeks in a 4x4 cross-over design. Each feeding period was separated by a one-week wash-out during which weathers were kept on pasture. During the last 4 days of each feeding period, animals were individually kept in a metabolic cage, which was introduced into a respiration chamber to measure feed and water intake, gas exchange, fecal and urinary excretions. Feed and feces samples were analyzed for N and aNDFom according to VDLUFA III and Se in feed, feaces and urine by inductively coupled plasma - mass spectrometry. Feed and fecal gross energy was measured by bomb calorimetry. The statistical analysis was performed by R Statistical Software (v4.3.2; 2023). Data were analysed with a linear mixed model and pairwise differences between levels of fixed effects (feeding period, group and their interactions) were tested by using the Tukey Kramer test. Differences were considered significant at $P < 0.05$ and as a trend at $P < 0.1$.

Results: Weathers fed NIG had a higher Se digestibility and Se balance than wethers fed hay, SAL or TREM ($P < 0.05$). The OM digestibility was lower in TREM than hay ($P < 0.05$) but not between hay, NIG and SAL fed weathers. The CP digestibility of the NIG was comparable to the hay and greater than the SAL and TREM rations ($P < 0.05$). Relative to hay feeding, aNDFom digestibility was reduced with TREM ($P < 0.05$) and tended to be lower with SAL ($P < 0.1$) but not NIG inclusions. The energy digestibility was lower in the SAL and TREM than hay and NIG diets. The metabolizable energy (ME) of the ration containing SAL and TREM leaves was in average 2.6 MJ lower than ME of hay ($P < 0.05$).

Conclusions: NIG leaves are a suitable source of Se supply. None of the tree leaves compromise CP digestibility, but the reduction in OM, ME and energy digestibility with TREM and SAL leaf supplementation was potentially owed due to contained tannins.

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32.

Silage quality of multi-species forages with plantain, alfalfa and ryegrass in vacuum-packed mini-silos

Silagequalität von Gemengen aus Spitzwegerich, Luzerne und Weidelgras in vakuumverpackten Mini-Silos

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Due to climate change and the demand to increase sustainable production in conventional farming, the cultivation of fine-seeded legumes is moving more into focus. In combination with ribwort plantain (Plantago lanceolata), a drought-resistant, deep-rooting plant, multi-species forages increase biodiversity in pastures and fields and can be a possible adaptation to increasingly frequent and longer dry phases. As a grazing forage, plantain is known for its high digestibility and potential to reduce methane emissions and urinary nitrogen excretion, making it a promising forage species [1, 2]. Most studies testing plantain as a feed for ruminants used freshly cut plantain or plantain as part of the pasture, while studies examining preserved plantain are scarce [3]. Therefore, the aim of this study was to test the silage-ability and silage quality of swards with and without plantain.

Methods: The third cut of three different forages was investigated: grass (G), grass plus alfalfa (G+A), and grass plus alfalfa plus plantain (G+A+P). The following varieties and mixing ratios were used: G: 10% timothy Lischka, 30% meadow fescue Perseus, 30% perennial ryegrass Polim, 30% perennial ryegrass Kaiman; G+A: 37.5% alfalfa Plato, 37.5% alfalfa Verko, 15% timothy Lischka, 5% perennial ryegrass Polim, 5% perennial ryegrass Kaiman; G+A+P: 32.5% alfalfa Plato, 32.5% alfalfa Verko,15% timothy Lischka, 5% perennial ryegrass Polim, 5% perennial ryegrass Kaiman, 10% ribwort plantain (all from Camena Samen, Lauenau, Germany). They were planted in the Hessian State Domain Frankenhausen in May 2023, and harvested in late July 2024 using a mowing machine (each forage type from two paddocks each 1.5×14 m^2). The harvested material was chopped using a forage chopper to a length of 3 to 10 cm and wilted overnight at room temperature before being ensiled. A total of 5 kg of chopped forage for each type was packed into vacuum bags (250 × 400 mm), with each bag containing approximately 300 to 500 g. The air was evacuated, and the bags were sealed using the vacuum-packing machine (Caso FastVac 500, CASO GmbH, Arnsberg, Germany). The bags were then stored at room temperature for 53 days. When bags were opened on average 3.8 kg of each type were pooled together for analysis. The nutrient contents, energy content, pH and fermentation parameter were analysed with near-infrared spectroscopy (NIRS) at the Agricultural Analysis and Research Institute Nordrhein-Westfalen (LUFA NRW). The metabolizable energy (ME) content was calculated based on GfE 2008 for ruminants.

Results: Ensiling the prewilted materials resulted in dry matter (DM) contents of 36.1, 27.0 and 24.5 % for G, G+A, and G+A+P, respectively, with pH values of 4.3, 4.6 and 4.6, respectively, and lactic acid concentration of 33.5, 39.2 and 27.1 g/kg DM. The acetic acid concentration increased with alfalfa and plantain, being 18.8, 32.9 and 36.5 g/kg DM for G, G+A, G+A+P. The crude protein contents of G, G+A, and G+A+P silages were 11.8, 17.4 and 14.0 % of DM. The NDF content was highest in G silages with 49.0% of DM and decreased with addition of legume (45.9% DM) and forb (35.7 % DM). Predicted ME contents of G, G+L, and G+L+P were 9.8, 10.0, 9.7 MJ/kg DM.

Conclusions: One adaption strategy to increasing periods of drought is the use of multi-species forages, including deep rooted species like alfalfa and plantain on fields and pastures. In order to plan rations for ruminants reliably, it is important to evaluate the feed value of these mixtures also of their preserved forms like silages. Our first results indicate a high nutritive potential of mixed forages containing plantain. However, the strong odour of this silage might influence the palatability. Further research is needed to evaluate the digestibility and acceptability by animals.

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33.

In vitro fermentation kinetics of different herbs in response to polyethylene glycol addition

In-vitro-Fermentationskinetik verschiedener Kräuter mit und ohne Zusatz von Polyethylenglycol

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Herbs can play a crucial role in ruminant nutrition. Aside from aspects like being more drought-tolerant compared to grasses, herbs also contain a variety of secondary plant compounds, like essential oils, saponins, flavonoids, and tannins. Tannins can complex proteins and protect them from breakdown in the rumen. They have the potential to modulate rumen fermentation in a positive way but can also have adverse effects and can, source and dose dependent, reduce feed intake and digestibility [1]. Polyethylene glycol (PEG) is a tannin-binding agent that can neutralize the detrimental effects of tannins. This study evaluates the fermentation characteristics of various herbs, examining their *in vitro* gas production rates in response to PEG supplementation.

Methods: A total of 32 herb samples, representing seven species in different varieties (accessions): *Lotus corniculatus* (7 accessions), *Medicago lupulina* (4 accessions), *Plantago lanceolata* (7 accessions), *Achillea millefolium* (4 accessions), *Cichorium intybus* (4 accessions), *Carum carvi* (4 accessions), and *Hedysarum coronarium* (2 accessions), were supplied by P.H. Petersen Saatzucht Lundsgaard GmbH (Grundhof, Germany). The samples were freeze-dried and ground through a 1-mm sieve. *in vitro* gas production was determined using the Hohenheim gas test [2]. Approximately 200-250 mg dry matter (DM) of each sample was weighed into 100 ml calibrated glass syringes. The rumen fluid was obtained from two ruminally cannulated, non-lactating Holstein Friesian cows, maintained on a high-forage diet. For each sample, duplicate syringes with and without addition of PEG (500 mg) were incubated in two runs on separate days (n=4 per treatment). The samples were incubated at 39 °C. Gas production was recorded after 4, 8, 12, 24, 32, 48, 56, and 72 h of incubation. The data were fitted to the model of Ørskov and McDonald [3] using R studio (version 4.3.3). The model (y = a + b [1- e-ct]) included the following variables: a=gas production from the immediately soluble fraction (ml/200 mg DM), b=gas production from the insoluble fraction (ml/200 mg DM) (a + b represents potential maximal gas production), c=gas production rate constant for the insoluble fraction (h^{-1}), t=incubation time (h), and y=gas produced at a time "t". The experiment included various species, with samples tested across multiple accessions to examine intra-species variability. Non-linear least squares models (nls) were used to estimate fermentation parameters, and comparisons were made across species and accessions using ANOVA. Statistical significance was determined at $P < 0.05$.

Results: We detected differences in gas production between herb species ($P < 0.05$). The highest corrected 24-h gas production was recorded for Cichorium intybus (50.3± 4.28 mL/200 mg DM), while the lowest gas production was observed for Achillea millefolium (32.7 ± 0.72 mL/200 mg DM). After 4 h, Cichorium intybus had again the highest (19.5 ± 1.53 mL/200 mg DM) and Achillea millefolium (11.6 ± 0.36 mL/200 mg DM) the lowest gas production. These patterns extended to the potential gas production (a+b) and gas production rate (c), with Cichorium intybus consistently having the highest values among all species, while Achillea millefolium had the lowest. The addition of PEG enhanced both the potential gas production (a+b) and the fermentation rate (c) in Carum carvi, Hedysarum coronarium, Lotus corniculatus, and Cichorium intybus.

Conclusions: The addition of PEG resulted in an increased fermentation rate and gas production in several species, likely those with relevant tannin contents such as Lotus corniculatus. This demonstrates the effectiveness of PEG to mitigate the inhibitory effects of tannins on microbial fermentation. The observed differences between species and within species highlight the importance of selecting appropriate herb types based on their potential fermentation characteristics and tannin content.

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34.

Ruminal crude protein degradation measured *in sacco* and *in vitro* by co-incubation of *Streptomyces griseus* protease and carbohydrase

Ruminaler Rohproteinabbau gemessen in sacco und mittels Co-Inkubation von Streptomyces griseus Protease und einer Carbohydrase

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The *Streptomyces griseus* protease (SGP) is used in SGP-method (SGPM) to estimate rumen undegraded protein in feedstuffs *in vitro* [1]. However, feed specific-protein-carbohydrate complexes require additional carbohydrases in SGPM to reduce differences to *in sacco* crude protein (CP) degradation data [2]. Recently published data have shown that SGP and a carbohydrase can be co-incubated under the conditions set by the SGPM without significantly reducing the activity of the carbohydrases [3]. The objective of the recent study was to investigate the influence of carbohydrases on ruminal CP degradation determined in SGPM with reference to *in sacco* CP degradation data.

Methods: The *in sacco* CP degradation data (reference) of rapeseed meal, dried distillers‘ grains with solubles, wheat grains, maize grains, grass silage, maize silage and pea silage was determined using three rumen-fistulated Holstein dairy cows. The SGPM was conducted according to Licitra et al. [1]. In brief, duplicates of each 0.5 g of ground material were pre-incubated for 1 h at 39 °C in 40 mL of borate-phosphate buffer (pH 6.75) with 0.5 mL Penicillin-Streptomycin solution (Thermo Fisher Scientific, Massachusetts, USA) under continuous shaking. After pre-incubation, the carbohydrase α-amylase (A) (“Termamyl 2x”, Univar Solutions, Essen, Germany) or Viscozym® L (V) (cell wall-degrading enzyme mixture consisting of ß-glucanase, cellulase, hemicellulase, arabanase and xylanase, Merck KGaA, Darmstadt, Germany) were each added in four doses: 0.1 mL (A_1), 0.2 mL (A_2), 0.4 mL (A_3), 0.8 mL (A_4), 0.188 mL (V_1), 0.375 mL (V_2), 0.750 mL (V_3) and 1.5 mL (V_4). The SGPM without A and V was used as control (C). Subsequent after adding carbohydrases, the SGP solution (0.58 U/mL) was added at a ratio of 24 U/g feed true protein (CP minus non-protein nitrogen as fraction A). Incubation times were 0, 2, 4, 8, 24 and 48 h. After incubation, the solutions were filtered, residues dried and analysed for Kjeldahl nitrogen. Protein degradation parameters were estimated in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and used to determine effective CP degradation (ED, %/CP) for assumed ruminal passage rate of 0.05/h (ED_5). Statistical analysis of *in sacco* and *in vitro* ED_5 was performed in SAS 9.4 in MIXED procedure. Different lower-case letters indicate significant differences ($p < 0.05$) of least squares meansbetween ED_5 estimated by SGPM and SGPM with A or V, respectively.

Results: The *in sacco* ED_5 (%/CP) were underestimated for all feedstuffs in SGPM without and with A and V, respectively ($p < 0.05$). The ED_5 estimates in the sequence *in sacco*, C/ A_1, A_2, A_3, A_4/ V_1, V_2, V_3 and V_4 were: rapeseed meal 78, 60^b/ 60^b, 59^b, 59^b, 59^b/ 62^a, 61^a, 63^a and 62^a; dried distillers‘ grains with solubles 91, 55^b/ 55^b, 55^b, 55^b, 55^b/ 56^a, 56^a, 55^b and 54^b; wheat grains 89, 67^b/ 69^a, 70^a, 70^a, 72^a/ 72^a, 74^a, 74^a and 78^a; maize grains 74, 24^b/ 26^b, 26^a, 26^a, 26^a/ 25^a, 25^a, 25^b and 25^a; grass silage 86, 68^b/ 68^b, 68^b, 67^b, 67^b/ 69^a, 69^a, 69^a and 68^b; maize silage 89, 64^b/ 66^a, 66^a, 66^a, 67^a/ 67^a, 67^a, 67^a and 66^a; pea silage 87, 78^b/ 79^b, 79^b, 78^b, 79^b/ 81^a, 81^a, 81^a and 82^a.

Conclusions: The co-incubation of α-amylase or a cellulolytic enzyme mixture with SGP did not reduce the differences between *in sacco* and *in vitro* ruminal crude protein degradation. The incubation conditions and enzymatic interactions might lead to insufficient degradation of protein-carbohydrate complexes. Pre-incubation of carbohydrases prior to the SGPM may be more promising as recommended specific incubation conditions of the carbohydrase in question can be considered.

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35.

Comparison of energy supply for dairy cows calculated from the net energy for lactation and metabolizable energy system in Germany based on initial data from a feeding trial

Vergleich der anhand des Nettoenergie Laktation und umsetzbaren Energie Systems in Deutschland berechneten Energieversorgung von Milchkühen auf Basis erster Daten eines Fütterungsversuchs

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Recent studies on energy metabolism of dairy cows have shown that the recommendations on energy supply given by GfE (2001) [1] on the basis of net energy for lactation (NEL) no longer correspond to the current state of knowledge (GfE, 2023) [2]. Therefore, initial data from a trial with dairy cows was used to compare the recommendations on energy supply according to [1] and [2].

Methods: The study employed a total of 48 lactating German Holstein cows which were assigned to 4 groups in 3 consecutive periods, according to a 2x2 factorial design, resulting in 12 different feeding groups. Each period consisted of 3 weeks of adaptation and 3 weeks sampling phase. The factors were concentrate feed proportion (CFP) and feed composition. CFP was set at 30% and 55% of dry matter (DM) intake. Feed composition varied in regard to different roughage and concentrate components. Feed was offered *ad libitum via* weighing throughs, automatic concentrate feeders (Insentec B.V., Marknesse, The Netherlands) and the Greenfeed system (C-Lock Inc., Rapid City, SD, USA). Feed and rectal faecal samples were collected twice a week, pooled over 3 weeks, dried and analyzed for acid insoluble ash (AIA) and chemical composition. Digestibility of crude nutrients and organic matter was determined using AIA as a marker. Feed intake level (FIL) was calculated according to [2]. According to [1], metabolizable energy content of the ration was calculated using equations based on the nutrient content and *in vitro* parameters ($ME_{2001\_in\_vitro}$) as well as the *in vivo* derived digestible crude nutrients ($ME_{2001\_in\_vivo}$). This was followed by determination of NEL ($NE_{Lin\_vitro,\ NELin\_vivo}$). Organic matter digestibility was calculated using equations according to [2] and determined *in vivo*. Both were used within the 3-step procedure [2] to calculate metabolizable energy ($ME2_{023\_in\_vitro\_FIL1}$, $ME_{2023\_in\_vivo}$). Equations for correcting $ME_{2023_in_vitro_}$FIL1 by FIL were applied ($ME_{2023\_in\_vitro\_FILi}$). Data were analyzed with a linear model (LM) using RStudio (version 4.3.2). Lin's concordance correlation coefficient (CCC) [3] was determined to evaluate concordance between calculated energy contents derived from the different evaluation systems.

Results: Energy content of the rations ranged from 6.6 to 7.4 MJ/kg DM for NEL_{in_vitro}, from 10.8 to 12.0 MJ/kg DM for $ME_{2001\_in\_vitro}$ and from 11.1 to 12.6 MJ/kg DM for $ME_{2023\_in\_vitro\_FIL1}$, respectively. CCC for $ME_{2001\_in\_vitro}$ and $ME_{2023\_in\_vitro\_FIL1}$ was 0.45 and LM resulted in y = 1.02x + 0.32. Intercept was not significantly different from 0 (p=0.57). Mean FIL was 3.5 which led to an average reduction in energy content of 1.0 MJ ME/kg DM resulting in a range from 10.2 to 11.4 MJ/kg DM for $ME_{2023\_in\_vitro\_FILi}$. The energy contents based on *in vivo* digestibility measurements varied between 5.9 and 7.1 MJ/kg DM for NEL_{in_vivo}, between 9.9 and 11.7 MJ/kg DM for $ME_{2001\_in\_vivo}$ and between 9.9 and 12.1 for $ME_{2023\_in\_vivo}$, respectively. CCC for $ME_{2023\_in\_vitro\_FILi}$ and $ME_{2023\_in\_vivo}$ was 0.37 and LM resulted in y = 0.73x + 3.22 with an intercept significantly differing from 0 ($p<0.05$). CCC for $ME_{2001\_in\_vivo}$ and $ME_{2023\_in\_vivo}$ was 0.82. The corresponding LM yielded y = 1.14x – 1.24 and intercept was significantly different from 0 ($p<0.001$).

Conclusions: ME content of the rations fed was higher according to the new evaluation system [2] compared to the former one [1]; both when applying the *in vitro* equations at FIL 1 and when using data from digestibilities measured *in vivo*. $ME_{2023\_in\_vitro\_FILi}$ and $ME_{2023\_in\_vivo}$ matched better at higher energy contents of the ration than at lower ones. The data set of the experiment should be further examined to consider methane emissions and nitrogen excretion in relation to the energy losses. Also, energy balance of the dairy cows should be investigated applying the different evaluation systems.

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36.

Exploring exhaled volatile organic compounds as new indicators for the energy balance of dairy cows: A comparison with serum β-hydroxybutyrate

Untersuchung ausgeatmeter flüchtiger organischer Verbindungen als neue Indikatoren für die Energiebilanz von Milchkühen: Ein Vergleich mit Serum β-Hydroxybutyrat

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The energy status of dairy cows is commonly assessed by blood metabolites such as β-hydroxybutyrate (BHB) concentrations, which requires invasive sampling. Exhaled breath may represent a promising, low-invasive alternative as it contains volatile organic compounds (VOC) originating from the cow's endogenous metabolism. If the energy status of dairy cows influences exhaled VOC, and if so, to which extent they can reflect a status of energy deficit, compared to serum BHB concentrations, has not been studied yet. Therefore, the aim of this study was to assess the suitability of exhaled VOC to discriminate between negative (NEB) and positive energy balance (PEB) states of dairy cows, aiming at identifying discriminating exhaled VOC. Furthermore, the strength of correlations between energy balance (EB) and exhaled VOC should be compared to that of the correlation between EB and serum BHB.

Methods: Thirty-four lactating Holstein cows (milk yield: 37.77±7.41 kg/d, DIM: 33.2±15.1) were fed fresh herbage *ad libitum* for 6 weeks and supplemented with 5 to 6 kg/d concentrate. In week 1, 3 and 6, exhaled breath was sampled and analyzed for VOC according to [1], blood was collected from the vena jugularis and analyzed for serum BHB. The EB (MJ NEL; was expressed as such following current Swiss practice) was calculated as the difference between energy intake through feed and energetic output for maintenance and milk production. To discriminate between exhaled VOC profiles of NEB and PEB states of cows, 22 cows with a NEB in week 1 (-10.9 to -96.7 MJ NEL) and PEB in week 6 (5.00 to 60.9 MJ NEL) were selected. Partial least squares-discriminant analyses (PLS-DA) were performed using MetaboAnalyst (v.6.0). Models with a predictive ability parameter (Q2) > 0.5, goodness of fit (R^2) > 0.7 and error rate (ER) < 0.4 were considered valid. The most discriminating VOC were chosen based on a VIP score of >2 and a significant Wilcoxon's test between PEB and NEB samples ($P < 0.05$). To study the correlations between the calculated EB with both discriminating exhaled VOC and serum BHB concentrations, data of all 34 cows across all sampling weeks was used for repeated measures correlations (R; v. 4.3.3; package rmcorr). Differences in the strength of correlations were evaluated using the Williams Test (R; package cocor).

Results: Throughout this study, serum BHB concentrations ranged from 0.22 to 1.66 mmol/L and the calculated EB ranged from -87.9 to 33.1 MJ NEL. Serum BHB concentrations (NEB: 0.39 to 1.66; PEB: 0.22 to 0.60 mmol/L; $P < 0.01$) and exhaled VOC profiles (Q2=0.90, R^2=0.82, ER=0.33) differed between NEB and PEB states of cows. Three exhaled VOC, namely the fatty aldehydes octanal, nonanal and decanal, were found to be robustly discriminatory between NEB and PEB states of cows. Higher concentrations of exhaled octanal (+35%, $P < 0.01$), nonanal (+27%, $P = 0.01$) and decanal (+32%, $P < 0.01$) and serum BHB (+98%, $P < 0.01$) were found when the cows where in NEB states compared to PEB states. The EB was negatively correlated with exhaled octanal, nonanal, decanal ($r = -0.32$, $r = -0,49$, $r = -0.54$, respectively; all $P \leq 0.01$) and serum BHB ($r = -0.62$; $P < 0.01$). The strength of correlations of EB with serum BHB did not differ from those with octanal ($P = 0.24$) and decanal ($P = 0.45$) but from that with nonanal ($P < 0.01$).

Conclusions: In this study, exhaled VOC and serum BHB were suitable for discriminating between NEB and PEB states of cows. The EB showed a strong correlation with serum BHB concentration and similar correlations with the fatty aldehydes octanal and decanal. The discriminatory fatty aldehydes are likely formed during lipolysis via the fatty alcohol cycle [2]. The usability of the exhaled fatty aldehydes as biomarkers for EB has to be further investigated.

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37.

Effect of two liquid complementary feeds containing different energy sources on performance and BHB level in blood of fresh-milking Holstein Friesian dairy cows

Einfluss von zwei verschiedenen flüssigen energieliefernden Ergänzungsfuttermitteln auf die Leistung und BHB Werte im Blut von frischmelkenden Holstein Friesian Kühen

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The transit phase of high-yielding dairy cows is often accompanied by the occurrence of negative energy balance. In many cases, the animals show metabolic condition known as ketosis. The high degree of mobilization of the endogenous resources leads to an accumulation of ketone bodies in the blood and thereby increasing the risk of fatty liver. Negative effects of ketosis include reduced milk yield, low fertility rates, a higher incidence of metabolic disorders, such as abomasal displacement, and a shorter lifespan. During lactation there is an increased demand for lactose. Therefore oxaloacetate is not available for energy production. The compensation of the energy deficit cannot be guaranteed. In this case glucogenic precursors can serve as energy sources. The aim of this study is to investigate whether the different compositions of complementary feeds can achieve different levels of energy deficit compensation.

Methods: In this study 61 multiparous Holstein Friesian dairy cows were randomly assigned to one of two trial groups at the day of calving. The animals received either a product containing glycerol, propylene glycol and isomaltulose (CON, N=30) or a product containing propylene glycol, isomaltulose, glycerol, sodium propionate, choline chloride, niacin, cobalt and a flavouring agent (TRL, N=31). Products were applied by the liquid feeder in the automatic milking system starting with 50g/h/d increasing to 250g/h/d within 5 days p.p. and continuously with 250g/h/d until 60 days in milk. Initial data collection included the lactation number, the body condition score and calving difficulty. Health status of the cows was determined according to Huzzey et al. [1] post partum and weekly until day 60. The animals were categorized as follows :(1) „no disorder of interest“ including cows that did not have retained placenta, displaced abomasum, subclinical ketosis, lameness, postpartum retention, metritis, mastitis, pneumonia or death; (2) „one disorder“ included cows that developed only one of the aforementioned health disorders; (3) „more than one of the aforementioned disorders or death“. Calving difficulty was evaluated by three categories: (1) unobserved/ unassisted; (2) assisted by 1 person; (3) assisted by more than 1 person including veterinary help. Body condition score was defined according to Roche et al. [2]. The level of beta-hydroxy-butyrate (BHB) in blood (mmol/L) was measured using a BHB-Check device (Pharmakon GmbH, Deutschland) on lactation day 10, 20 and 30. BHB values between 1.3 and 1.8 mmol/L were evaluated as subclinical ketosis, while a BHB value above 1.8 mmol/L indicated clinical ketosis. Performance parameters included milk yield, milk fat (%), milk protein (%) as well as voluntary milkings per cow per day within the periods lactation day 1-30 and 31-60. Data were extracted from the automatic milking system (Lely A4). Initial data were statistically analysed by the chi-square test and the Mann-Whitney U test. Negative energy balance and performance data were evaluated by ANOVA analysis. The significance level was set at 95%. P-values < 0.05 indicate significant differences.

Results: Lactation number, health status, body condition score and calving difficulty did not differ between groups. BHB level on day 10 was 1.03 for both groups (p=0.975), 1.12 for CON and 0.97 for TRL on day 20 (p=0.133) and 1.04 for CON and 0.99 for TRL on day 30 (p=0.475). Average fat and protein corrected milk yield (FPCM) based on 4% fat and 3.4% protein between lactation day 1-30 was 41.5 kg for CON and 43.0 kg for TRL (p=0.381). Between lactation day 31 and 60 FPCM was 46.2 kg for CON and 49.6 kg for TRL (p=0.066). Average voluntary milkings between lactation day 1-30 were 3.14 for CON and 3.29 for TRL (p=0.301). Between lactation day 31 and 60 the voluntary milkings were 3.25 for CON and 3.50 for TRL (p=0.129).

Conclusions: No statistical differences were found between groups. There was a trend for higher FPCM between day 31 and day 60 for the TRL group.

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38.

Estimation of methane energy loss in horses based on experimental data from the legacy of O. Kellner and G. Fingerling (1931 – 1939)

Schätzung des Methanenergieverlustes beim Pferd basierend auf experimentellen Daten aus dem Nachlass von O. Kellner und G. Fingerling (1931 – 1939)

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The loss of methane energy (CH_4-E) derives from microbial fermentation of organic matter (OM), and hereby mainly of cell wall carbohydrates. Since in the hindgut reductive acetogenesis dominates over methanogenesis, the CH_4-E loss per unit of digestible energy is much lower in horses [1] than in ruminants, which use ruminal methane production as primary hydrogen sink. Despite the smaller amount of CH_4-E losses in horses, they need to be assessed and included in any energy evaluation system when based on metabolisable energy. This study aimed to derive an estimate for CH_4-E using data from horse trials from the legacy of O. Kellner and G. Fingerling (1931 – 1939) [2].

Methods: Data bases consisted of 60 diets tested in respiratory trials with non-working adult warmblood geldings (body weight (bwt) ≈ 550 kg) [2]. The following feedstuffs were investigated by the difference method with meadow hay, oat grains, linseed meal and molasses or meadow hay alone as core diet: straw of different botanical origin, meadow hay at different stages of vegetation, alfalfa and clover hay, oat grains, ground corn, rye bran, wheat and rice gluten, fodder beets, sugar, potato flakes, potato starch, horse beans, dried distillers' grains, cocoa shells, and peanut oil. The feedstuffs had been analytically characterised inter alia by crude ash, crude protein (CP), crude lipids (CL) and crude fibre (CF). Since data of starch and sugar contents were not provided, they were taken from diverse feed tables such as [3]. The organic residue was calculated as follows: OR = OM – CP – CL – starch+sugar. Dry matter (DM) intake and dietary nutrient contents were as follows: (mean ± standard deviation (min-max)); 52 ± 7.2 (42 – 70) g/ $kg^{0.75}$ bwt; in g/kg DM, CP 127 ± 19,0 (102 – 237), CL 53 ± 12,4 (23 – 107), CF 250 ± 41,2 (173 – 361), starch+sugar 156 ± 58,7 (66 – 299), OR 596 ± 62,8 (486 – 736). CH_4-E losses were related to either CF or OR in the feed. Data were characterised by arithmetic mean ± standard deviation and linear regression analyses were performed to evaluate nutrient impacts on CH_4-E.

Results: The mean CH_4-E loss related to CF or OR was 1.99 ± 0.38 kJ/g CF and 0.83 ± 0.16 kJ/g OR (SD% = 19 for both). The loss of kJ CH_4-E/g OR or CF was independent from both intake and dietary concentration of OR or CF and DM intake as well ($P > 0.05$).

Conclusions: The CH_4-E loss in horses can be estimated with sufficient accuracy for energetic feed evaluation from dietary CF or OR concentration. The first confirms earlier evaluations which included additional data also from growing horses and broad mares in pregnancy and lactation [1], the second opens the possibility for energy evaluation systems without the use of CF. Since no effect of DM intake on CH_4-E per g of CF or OM was observed within the intake range studied, it might be hypothesised that CH_4-E is not or to a small extent only affected at higher feed intake.

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39.

Comparison of different methods for estimation of the daily amount of urine excreted by lactating and dry Holstein cows

Vergleich verschiedener Methoden zur Schätzung der täglich ausgeschiedenen Harnmenge von laktierenden und trockenstehenden Holstein Kühen

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A key factor to calculate nitrogen excretion of dairy cows is the knowledge of the amount of daily excreted urine. Creatinine is considered a reliable marker for estimation of urine volume as it is not metabolised, secreted or reabsorbed by the kidneys and is unaffected by water intake. Based on these assumptions, two methods based on balance experiments with known urine and creatinine excretions were developed to estimate urine volume based on urinary creatinine concentration for later use with urine spot samples. With the first method, the daily excreted amount of creatinine was linearly regressed on body weight. The slope of the regression was used to estimate urine volume for spot samples as described earlier. The second method includes the non-linear regression of urine volume on creatinine concentration in urine aimed at direct future estimation of urine volume without knowledge of body weight. Both estimation methods were compared to the standard method of determination of urine volume from balance trials using Lin's concordance correlation coefficient (CCC) [1] and Bland-Altman-Plots.

Methods: The dataset is based on 9 balance trials, which were conducted at the experimental animal facility in Brunswick with a total of 47 lactating and 7 dry Holstein cows. Trials involved quantitative urine collection via urine devices over five consecutive days. Cows were housed in a heated barn and tethered with a rope around their necks. They had *ad libitum* access to fresh water in individual drinking bowls. Feeding strategies adhered to experimental designs, either with set feed amounts at 5:30 AM and 3:00 PM or *ad libitum* access to feed. Feed supply was calculated according to the recommendations of the GfE [2]. For lactating cows, the basal diet contained maize silage with proportions of concentrates of 30% or 40% and crude protein contents of 131 – 142g/kg dry matter (DM), depending on the different experiments. Dry cows' basal diet contained 50% of maize silage and 50% of grass silage with mineral feed concentrates and crude protein contents of 108g/kg DM. Urine samples underwent analysis for creatinine concentration using an HPLC system (Shimadzu, Kyoto, Japan). Statistical analyses were performed using RStudio with R (version 4.3.0) and linear regressions with base R. CCC was calculated to evaluate accuracy and precision of the method comparison. Bland-Altman plots were created with package BlandAltmanLeh (version 0.3.1) to compare quantitatively collected urine amounts to estimated values obtained from the two estimation methods.

Results: A CCC of 0.81 with the standard method of urine collection is estimated for the linear urine volume estimation method using body weight and creatinine amount whereby the Bland-Altman-Plots suggest a systematic difference between both methods of 4.3 L/d. Lower confidence limit of differences is –16.2 and upper limit 24.9 L/d. For the non-linear urine volume estimation method using creatinine concentration a CCC of 0.85 with the standard method of urine collection is determined. Bland-Altman-Plots indicate a systematic difference of 0.1 L/d between both methods. Lower confidence limit of differences is –15.8 and upper limit 16.0 L/d.

Conclusions: Both methods for estimating urine volume lead to reliable and reproducible good results, which conclude in a usability of both methods for spot urine samples.

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40.

Impacts of harvest weights of *Tenebrio molitor* on amino acid digestibility and metabolisable energy in caecectomised laying hens

Auswirkungen von Erntegewichten von Tenebrio molitor auf die Aminosäurenverdaulichkeit und die Umsetzbare Energie bei caecectomierten Legehennen

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Yellow mealworm larvae (*Tenebrio molitor*; TM) have recently attracted attention as a protein and energy source in poultry diets. There is an inexplicable variation in the nutritive value of TM larvae for poultry beyond known influences, such as rearing conditions and feeding substrates [1]. In addition, there is little information on the nutritive value of TM pupae for laying hens. Therefore, this study investigated the influence of harvest weights of TM larvae and pupae on the nutrient composition, amino acid (AA) digestibility, and nitrogen-corrected metabolisable energy (MEN) in caecectomised laying hens.

Methods: Six experimental diets were formulated to meet or exceed recommendations for laying hens with a daily egg production of 60 g and a body weight of 1,800 g [2]. Each diet contained 250 g maize starch/kg or 250 g/kg of one of five partly defatted TM meals. These ingredients were added to complement a premixture of ingredients common across all diets, mainly containing maize, soybean meal, wheat gluten, limestone, and soybean oil. The TM meal variants comprised average insect-excrement-free body weights of larvae of 60 mg, 80 mg, 100 mg, and 120 mg, and pupae weight of 125 mg (P125). The TM variants were defatted using a mechanical screw press and dried to a dry matter (DM) content of ~955 g/kg. Diets were pelleted through a 3 mm die without steam. The study employed a 6×6 Latin square design, with 6 caecectomised laying hens fed the 6 diets over 6 periods. Hens were housed individually in metabolism units for 8 days/period and daily received 115 g of feed. Excreta were quantitatively collected during the last 4 days of each period. AA digestibility excluding basal endogenous losses was determined using a regression approach [3], where AA digestibility represents the slope of the linear regression of AA intake against the amount of AA digested. MEN was calculated using the difference method. Data were evaluated using the MIXED procedure in SAS.

Results: Crude protein (N×6.25) content was similar among the larvae variants (734–760 g/kg DM), but lower in P125 (676 g/kg DM) while crude fat was higher in P125 (216 g/kg DM) than in the larvae variants (95–124 g/kg DM). Similar to crude protein, AA contents were lower in P125 than in the larvae variants. Differences in the AA profile were small with values for the first-limiting AA (in g/16 g N) of 1.2 for methionine, 0.6–0.8 for cysteine, 5.1–5.3 for lysine, and 3.7–3.9 for threonine. TM harvest weight had no effect on the AA digestibility among the larvae variants for any AA. The AA digestibility of P125 was numerically highest among all TM variants. The average digestibility of the larvae variants and P125 was 94.8% vs. 97.0% for methionine, 87.2% vs. 92.9% for cysteine, 90.9% vs. 94.5% for lysine, and 89.4 vs. 93.9% for threonine. Differences between pupae and larvae were significant for alanine, histidine, isoleucine, lysine, serine, tyrosine, and valine ($p<0.05$). The crude fat digestibility was 95.5–97.5% and was unaffected by treatment. The MEN did not differ among larvae variants (18.1–18.9 MJ/kg DM), but ME_N of P125 was higher (20.9 MJ/kg DM, $p\leq0.02$).

Conclusions: There was no implication of an influence of the larvae harvest weight on AA profile, AA digestibility, and ME_N, while the results indicated a higher AA digestibility in pupae than in larvae. Therefore, feeding TM pupae instead of larvae may reduce nitrogenous emissions of egg production if the total AA content of the feed is reduced under consideration of the higher digestibility, depending on additional emissions of TM production up to the pupal stage. The lower AA as well as higher crude fat and ME_N contents of pupae than of larvae may originate from a less efficient defatting process, which was optimised for larvae.

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41.

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

Die Auswirkungen einer Glutaminsupplementierung auf das Wachstum, die Organmasse und den Glutaminstoffwechsel von Saugferkeln mit niedrigem Geburtsgewicht

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Low birthweight (L) pigs have impaired growth and small intestine (SI) development compared to normal birthweight (N) pigs [1]. We reported previously that L pigs supplemented with glutamine (Gln) had improved bodyweight (BW) and jejunal villus height compared to alanine supplemented L pigs [2]. However, it is not known if Gln supplementation alters SI mass, amino acid (AA) metabolism or the metabolic fate of Gln. Therefore, this study aimed to measure piglet growth, SI mass, duodenal AA profiles and Gln metabolism in L suckling pigs supplemented with Gln.

Methods: At birth, 50 male pigs from parity 2–9 sows with litter sizes of 15–20 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, and experimental pigs assigned to Gln (1 g/kg BW/days of age (d)) or a water (W) supplementation groups (L-Gln, L-W; n = 12 / group, N-Gln, N-W; n = 14 / group). Pigs were orally supplemented with 33% of their daily Gln dose suspended in water or an equal volume of water at 7, 12, and 17 h, from 2 to 19 d. Bodyweight was recorded and average daily gain calculated. At 1, 2, 8, 15 and 20 d, crump-rump length and abdominal circumference were recorded, and ponderal index (PI) and bodymass index (BMI) calculated. Prior (1.5 h) to slaughter (20 d), all pigs were given an oral bolus of 10 mg/kg BW 13C5-Gln to trace Gln metabolism. Blood samples were collected at birth and 4 h post birth to measure plasma metabolites and free AA profiles, and at slaughter to measure glutathione concentrations in red blood cells (RBC) and plasma enrichment of $^{13}C_5$-Gln, $^{13}C_3$-glucose (Glc) and $^{13}C_2$-Glc to assess gluconeogenesis from Gln. At slaughter, SI tissue length and empty weight was measured and the duodenum tissue sub-sampled to measure total AA and $^{13}C_5$-Gln enrichment. Data was analysed using the GLIMMIX procedure of SAS with repeated measures where required.

Results: From 17 to 20 d, N-Gln were heavier than N-W. Average daily gain was higher in N-Gln than N-W and in N than L pigs ($P < 0.05$). From birth to 20 d, L pigs were thinner, shorter ($P < 0.001$) and lighter ($P < 0.05$) than N, whilst BMI was lower at 2, 8 and 20 d in L-Gln than N-Gln ($P < 0.05$), and at 8, 15 and 20 d in L-W than N-W ($P < 0.05$). At 8 d, L-Gln were longer ($P = 0.03$), at 15 d they had a higher BMI ($P = 0.01$), and at 2 and 15 d their PI was higher than L-W ($P < 0.05$). At birth, the concentrations of five plasma FAA, citrulline and β-alanine were lower and carnosine and 1-methyl-histidine ($P < 0.05$) were higher, and the metabolites albumin ($P < 0.001$) and fructose ($P = 0.04$) were lower and lactate and inositol ($P < 0.001$) were higher in L than N pigs. At 4 h post-birth, levels of twelve plasma FAA, essential AA and ornithine were higher ($P < 0.05$) and glutamate and carnosine ($P < 0.05$) lower, whilst the metabolites albumin, glucose ($P < 0.001$) and total protein ($P = 0.004$) were lower, and inositol ($P < 0.001$) higher in L than N pigs. At slaughter, duodenum ($P < 0.002$), caecum ($P = 0.001$) and SI ($P < 0.001$) were heavier and the SI longer ($P < 0.003$) in N than L pigs. The stomach was heavier ($P = 0.004$) and duodenum longer ($P = 0.018$) in N-Gln than L-Gln pigs, whilst the SI was heavier ($P = 0.015$) in N-Gln than N-W pigs. Duodenal concentrations of total proline ($P = 0.03$) were lower in N-Gln than N-W. Plasma enrichment of $^{13}C_2$-Glc was higher in L-Gln than L-W ($P = 0.04$).

Conclusions: At birth, high concentrations of plasmainositol are a biomarker of L in multiple species, and coupled with higher carnosine; indicate an underdeveloped glucose metabolism in L compared to N pigs used in this study. Supplementation with Gln altered L pig Gln metabolism compared to water supplemented L pigs, with no effect on BW or SI growth. Gln supplementation improved N pig BW, average daily gain and SI weight compared to water supplemented N pigs. These results show Gln only improved suckling N pig growth and SI development.

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42.

Investigating the impact of gradual dietary reduction of soybean meal and crude protein content on the global warming potential of piglet feeds

Untersuchung der Auswirkung einer schrittweisen Reduktion des Sojaextraktionsschrot- bzw. Rohproteingehalts auf das Treibhausgaspotenzial von Ferkelfutter

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Feed production is a major cause of environmental pollution related to animal rearing systems as well as emissions from manure and fields [1]. Modulating the feed, e.g., lowering the dietary crude protein (CP) or soybean meal (SBM) content, can be a key strategy to reduce the environmental impact. This study aims to demonstrate that the gradual reduction of SBM in connection with a low CP content will have no negative influence on the growth performance of piglets under commercial conditions but will lead to improvements in sustainability parameters, in particular in a reduced global warming potential (GWP) of the feed.

Methods: A total of 384 piglets (Topigs x Pietrain; mixed sexes) were divided into four treatments with 12 pigs per pen and 8 repetitions. Piglets of the control treatment (CON) received a commercial diet which consisted mainly of wheat, barley, and SBM (about 14.5 %, 19 % and about 18 % in phase 1, 2 and 3, respectively). In treatment 2 ($T_{-25\%SBM}$) and treatment 3 ($T_{-50\%SBM}$) the SBM content was reduced in comparison to the control diet by 25 % and 50 %, respectively. In treatment 4 (T_{CP}) the SBM content was reduced by 50 % and the dietary CP was decreased by 1 %-point in all feeding phases. Crystalline amino acids were used to fulfil the animals' requirements and to keep the amino acid level constant between treatments. In addition to the performance parameters (body weight, weight gain, feed intake, FCR), the environmental impact of feed production was investigated through incorporating databases such as Global Feed LCA Institute (GFLI) in order to enable an accurate and customized environmental impact analyses. This study set the system boundary from cradle-to-feed gate and following the PEF Standard for feed producing animals. The statistical analyses were performed using the SAS statistical software package version 9.4 (SAS Inst., Cary, NC, USA). Mean values, as well as the standard deviation of the mean (SD), the least square mean (LSM) and the standard error of the mean (SEM), were calculated for all parameters with the procedure MEANS. All statements of statistical significance were based on $P < 0.05$.

Results: Neither a SBM reduction, nor an additional CP reduction had a significantly negative effect on the final body weight in comparison to the control group. The highest final body weight (BW) was achieved in $T_{-25\%SBM}$ (30.0 kg), followed by CON (29.5 kg), $T_{-50\%SBM}$ (28.9 kg) and TCP (28.2 kg), respectively. The feed intake over the total trial period varies between 34.1 kg (T-50%SBM) and 35.6 kg (T-25%SBM) but did not differ statistically significant between treatments.The GWP of the CON diet was calculated to be 1293 g CO_2 eq./kg feed, 1260 g CO_2 eq./kg feed and 1153 g CO_2 eq./kg feed, in phase 1-3, respectively. With each reduction of 25 % SBM in the diets, a decrease of about 10 to 13 % in GWP has been observed. The strongest reduction in GWP in comparison to the control diet was realized in the group which received the diet with a strongly reduced SBM (-50%) and a 1% CP reduction (T_{CP}: -21.2 %, -27.9 % and -25.4 % CO_2 equivalents in phase 1-3, respectively). However, the CP reduction itself (T_{CP} against $T_{-50\%SBM}$) only accounts for a GWP reduction of 2.33 %, 2.81 % and 1.62 % in phase 1, 2 and 3, respectively.

Conclusions: Piglets maintain their performance regardless of a reduced SBM or dietary CP level. Synthetic amino acids in protein- and soy reduced diets therefore allow a noteworthy environmental relief.

Acknowledgement: The study was financially supported by CJ Europe GmbH.

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43.

Influence of luminal D-galactose on L-alanine uptake in the jejunum of broiler chickens

Einfluss luminaler D-Galaktose auf die L-Alanin-Aufnahme im Jejunum von Masthühnern

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High luminal D-galactose concentrations impede the uptake of L-alanine (L-Ala) in the jejunum (JEJ) of broiler chickens [1]. To identify the underlying mechanisms, the present study aimed to determine the dependence of L-Ala uptake on the transepithelial potential difference (PDt) in the presence of high luminal D-galactose concentrations.

Methods: A total of 24 male broiler chickens (Cobb500) received a three-phase, wheat-based diet that was limited in crude protein (-2 % points of Cobb500 recommendations) for four weeks. Afterwards, chickens were slaughtered, and the JEJ was prepared for Ussing chamber experiments. Uptakes of [^{3}H]-L-Ala were measured in the presence of 100 mM of luminal D-mannitol or D-galactose. Uptakes were determined for three PDt (open circuit, 0 mV, -50 mV), each in the presence vs. absence of mucosal sodium (Na^+). Data were analysed using two-way repeated measures (RM) ANOVA comparing the factors "potential difference" and "sodium" for each hexose tested. Results are presented as least square means ± 95 % confidence interval.

Results: When comparing the factors "sodium" and "potential difference", two-way RM ANOVA revealed that L-Ala uptakes in the presence of luminal D-mannitol were significantly higher in the presence vs. absence of mucosal Na^+ (1.06 ± 0.063 vs. 0.83 ± 0.063 nmol·cm^{-2}·min^{-1}; $P < 0.001$), while the factor "potential difference" had no effect and no two-way interaction was found ($P > 0.05$). In the presence of luminal D-galactose, a significant "potential difference" × "sodium" interaction was found ($P < 0.05$). Further dissection of the interaction revealed, that L-Ala uptakes in the presence of Na^+ did not differ significantly under open circuit vs. 0 mV conditions (0.97 ± 0.10 vs. 0.83 ± 0.10 nmol·cm^{-2}·min^{-1}; $P > 0.05$), while clamping the PDt to -50 mV resulted in significantly lower L-Ala uptakes in the presence of mucosal Na^+ (0.59 ± 0.11 nmol·cm^{-2}·min^{-1} ; $P < 0.05$). The latter uptake was not different to the uptake at -50 mV in the absence of Na^+ (0.52 ± 0.10 nmol·cm^{-2}·min^{-1}; $P > 0.05$); whereas, uptakes of L-Ala were decreased by the absence of Na^+ under open circuit (0.57 ± 0.10 nmol·cm^{-2}·min^{-1}) and 0 mV conditions (0.53 ± 0.11 nmol·cm^{-2}·min^{-1}) ($P < 0.05$).

Conclusions: The decrease of L-Ala uptakes in the presence of Na^+ at -50 mV PDt due to the co-presence of 100 mM luminal D-galactose supports the assumption that subapical Na^+ accumulation could inhibit amino acid uptake in the co-presence of high luminal hexose concentrations.

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44.

Derivation of the protein and amino acid requirements of *Hermetia illucens* larvae

Ermittlung des Bedarfs an Protein und Aminosäuren von Hermetia illucens Larven

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The use of protein meal and fat from the larvae of *Hermetia illucens* as an alternative feed has increased in recent years, as insects grow faster, have a higher feed conversion rate and require less resources than pigs or poultry for growing. Currently, the requirement of most nutrients for maximum growth of larvae is largely unknown. Therefore, it is not yet possible to supply *H. illucens* larvae with a nutritionally optimized substrate. Currently, larvae are mostly fed with by-products from the food industry (fruit or grain residues). The aim of the present study was to estimate the requirement of *H. illucens* larvae for protein and essential amino acids for optimum weight gain. For this end, *H. illucens* larvae were fed semi-synthetic diets supplemented with different concentrations of either various sources of protein or various concentrations of essential amino acids.

Methods: Amounts of 30 g of 5-day old larvae were fed for 12 days semi-synthetic diets (consisting of corn starch, cellulose, fat, vitamin-mineral mixture) supplemented with various concentrations (g/kg diet: 0, 25, 50, 75, 100, 150) of isolated pea, soy, whey, or egg protein or various concentrations of essential amino acids (lysine, methionine, threonine, tryptophan, leucine, valine, isoleucine, arginine, phenylalanine, and histidine). In the experiment to determine the requirement of essential amino acids, the concentration of the amino acid to be tested were supplemented at levels of 0%, 25%, 50%, 75%, or 100% in relation to the concentration being in the diet supplemented with 100 g egg protein/kg diet. All other essential amino acids were supplemented at levels being in the diet supplemented with 100 g egg protein/kg diet. Each experiment was run with six replications. Data were analyzed using a Kruskal-Wallis test, and a broken-line model was used to estimate the requirement of the protein sources or essential amino acids for maximum growth rate.

Results: Greatest weight gains of the larvae during the rearing period were achieved with the various protein sources at the following supplementation levels (g/kg diet): Pea protein, 74.8; soy protein, 104; whey protein, 86.0; egg protein, 73.7. In the amino acid supplementation experiments, greatest weight gains were reached at the following supplementation levels of the individual essential amino acids (g/kg diet): lysine, 2.8; methionine, 1.8; tryptophan, 0.7; isoleucine, 3.0; phenylalanine, 3.0; histidine, 1.0; threonine, 3,3; valine, 5.6; arginine, 4.0; leucine, 2.1.

Conclusions: This study gives an indication of the requirement of *H. illucens* larvae for protein and essential amino acids for optimum growth during rearing. The data reported might be helpful for the optimization of feed substrates for *H. illucens* larvae rearing under practical conditions.

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45.

Dry matter intake, milk yield and milk composition of Holstein dairy cows in summer and autumn – a pilot approach to detect markers of heat stress

Trockenmasseaufnahme, Milchleistung und Milchzusammensetzung von Holstein-Kühen im Sommer und Herbst - ein Pilotansatz zur Identifizierung von Markern für Hitzestress

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Climate change places a significant burden on dairy farming, particularly through increasing ambient temperatures that can adversely affect the health and milk yield of dairy cows. Recently conducted controlled experiments in climate chambers have shown that short-term heat stress hinders the protein expression of key mammary gland enzymes, e.g. fatty acid synthase and casein synthase and reduce milk fat and protein concentrations [1]. The present study aims to investigate, whether similar changes in milk composition also occur under practical conditions, when Holstein dairy cows on milk producing farms are affected by seasonal heat periods in summer (SU; July, August) or not in autumn (AT; September, October).

Methods: A total of 35 multiparous Holstein dairy cows (2.3 parities, 138±89 days in milk (DIM), 35±3 kg milk/d in July 2023) from the herds in Oberholz-Leipzig (OHL) and FBN-Dummerstorf (FBN) were sampled for milk between July and October 2023. In this time period, cows were fed a total mixed ration (ME: FBN 10.0 MJ/kg dry matter (DM), OHL 11.2 MJ/kg DM; crude protein: FBN 133 g/kg DM, OHL 176 g/kg DM) *ad libitum*. Dry matter intake (DMI) and milk yield (MY) were recorded for 4 weeks in SU and AT, respectively, to calculate the weakly means. Ambient temperature and relative humidity (RH) were recorded in 15 min intervals by indoor sensors to calculate the weakly mean and temperature-humidity-index (THI). Individual milk samples were taken from the afternoon milking for the analysis of the milk composition in the 4th week of the SU and AT period, respectively. Before milk sampling, respiratory frequency (RF) was measured visually by counting the flank movement (breaths/min). Milk composition was analyzed by mid-infrared spectroscopy and flow cytometry. Milk fatty acids were analysed via lipid extraction and gas chromatography. Climate, DMI and MY data were analyzed with a repeated measure ANOVA using the MIXED procedure of SAS (version 9.4) with the fixed effects of season (SU, AT), location (OHL, FBN), time (week), and their interactions. Milk composition data was analysed with the fixed effects: season, location, and their interactions. Parity and DIM served as covariates.

Results: One week before milk sampling in SU and AT, respectively, mean ambient temperatures were in FBN 20.6°C (SU) vs 11.5°C (AT), RH 65.5% vs 89.1%, THI 66.4 vs 53.0 and in OHL 21.3°C vs 16.0°C, RH 67.8% vs 72.8% and THI 70.5 vs 60.0. Weekly DMI and MY were higher in SU than AT, whilst RF was higher in SU than in AT ($P<0.001$). Milk fat and protein percentages were lower in SU than in AT, whilst lactose percentage was higher in SU than in AT ($P < 0.01$). In OHL but not FBN, milk urea concentration was higher in SU than in AT ($P<0.01$). Somatic cell counts and milk fat/protein ratios were not altered between seasons. The milk fatty acid profile revealed lower percentages of C11:0, C13:0, C14:1cis-9, C16:1cis-9, C17:1cis-9, C18:3n-3 LNA, sum of polyunsaturated fatty acids (PUFAs) and of n-3 PUFAs in SU than in AT, whilst C18:0, C20:0, C22:0, C18:1trans-9 and C20:3n-6 were higher in SU than AT ($P<0.01$, respectively). No seasonal effects were found for the sums of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), n-6 PUFA or the ratio of the SFA sum and the PUFA sum.

Conclusions: The greater RF in SU indicates heat stress in cows. The higher DMI and MY in SU corresponds with the number of DIM and reflects the course of lactation, but if these variables were affected by THI needs to be validated. The lower milk fat and protein concentrations in SU do not mirror the course of lactation, suggesting a metabolic adaptation of the mammary gland to the higher THI. The increase in the portion of C18:0, C20:0 and C22:0 in SU could be explained by alterations in ruminal biohydrogenation, adipose tissue metabolism or mammary gland desaturases [2]. If saturated, long-chain milk fatty acids may serve as a marker of heat stress in dairy cows needs to be explored in future studies.

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46.

The influence of lameness in dairy cows on their methane emissions and methane related performance parameters

Der Einfluss von Lahmheit bei Milchkühen auf ihre Methanemissionen sowie ihre methanbezogenen Leistungsparameter

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The prevalence of lameness in dairy cow herds is approximately 22 %, with lame cows exhibiting lower milk production, increased veterinary expenditure and impaired reproduction [1]. To investigate the influence of the climate impact of lame cows, methane production, methane yield (methane production per kg dry matter intake; MY) and methane emission intensity (methane production per kg energy corrected milk yield; MI) from an ongoing feeding trial was retrospectively analyzed.

Methods: The trial proceeded over two consecutive years, lasting 18 weeks per year. The investigated herd consisted of 48 lactating German Holstein cows in each year, resulting in 77 investigated cows in total. Cows were in their first to fifth lactation and were fed according to the recommendations for energy and nutrient supply of the GfE [2] in a 2 x 2 factorial design. First factor was the concentrate level, and second factor included different feeding supplements, resulting in 4 differing rations, which changed every six weeks. Ruminal methane production of the cows was measured by the GreenFeed system (C-Lock Inc., Rapid City, SD, USA). The cows‘ water and feed intake were measured using weighing troughs and automatic feeders. Locomotion score was determined weekly according to Flower & Weary [3]. The cows were then retrospectively reassigned to 4 groups according to their locomotion score: healthy cows, slightly lame cows, lame cows and clearly lame cows. For the analyzation of the data, dry matter intake (DMI), energy corrected milk yield (ECM), daily methane production, MY and MI have been summarized to means for each cow in each week. Statistical calculation was carried out with R (Version 4.4.1) and R Studio (2024.04.2) using the package “nlme” to create mixed models. Locomotion score, lactation number, trial week and concentrate level were used as possible fixed effects and the random effect was selected as the animal with a random intercept and a random slope. An autoregressive covariance structure (corARMA) was applied in each model. Depending on the best fit, considering AIC and BIC the best model for each dependent variable was analyzed and selected. In each case the locomotion score turned out as an important fixed factor.

Results: Locomotion scoring resulted in 1219 weekly observations of healthy, 341 of slightly lame, 71 of lame and 14 of clearly lame cows, displaying a prevalence of lameness of 5 %. Healthy cows had a methane production of 495 ± 81 g/d (mean ± SD), DMI of 21.0 ± 2.8 kg/d and ECM of 31.6 ± 5.5 kg/d. Whereas clearly lame cows produced 427 ± 84 g/d methane, had a DMI of 15.4 ± 3.0 kg/d and an ECM of 24.8 ± 3.7 kg/d. Methane production was lower in the group of clearly lame cows compared to healthy cows ($p = 0.05$) and slightly lame cows ($p = 0.02$). Healthy, slightly lame and lame cows had similar daily methane productions. DMI of lame cows was significantly lower compared to healthy cows and ECM was significantly lower compared to healthy and slightly lame cows. Clearly lame cows had a significantly lower ECM and DMI in contrast to the other locomotion scores each. This resulted in a MY of 24 ± 4 g/kg DMI for healthy cows, 24 ± 4 g/kg DMI for slightly lame cows, 25 ± 4 g/kg DMI for lame cows and 27 ± 5 g/kg DMI for clearly lame cows and a MI of 16 ± 3 g/kg ECM in healthy cows, 16 ± 3 g/kg ECM in slightly lame cows, 17 ± 3 g/kg ECM in lame cows and 18 ± 5 g/kg ECM for clearly lame cows, respectively. MY and MI of clearly lame cows are significantly higher compared to the other locomotion scores each. Lame, slightly lame and healthy cows show similar MY and MI.

Conclusions: Although the methane production of clearly lame cows appears to be lower, lameness had a distinct impact on the cows’ performance parameters displayed by DMI and ECM and subsequently rose their MY and MI. More data of the ongoing study is needed to get bigger groups of lame and clearly lame cows to be able to access interactions of the fixed effects.

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47.

ProBioHuhn: Temporal and spatial variation of gut microbiota in organic chicken farms in Germany

ProBioHuhn: Zeitliche und räumliche Variabilität der Darmmikrobiota in ökologischen Hühnerfarmen in Deutschland

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The gut microbiota plays an important role in chicken's performance, immunity and behavior [1]. Host-related factors and environmental conditions are known to be key determinants in the development of the microbiota [2]. While knowledge of organic chicken farming continues to grow, there is still limited understanding of the variation in the gut microbiome of organic chickens. This study aims to investigate the difference in gut microbiome across organic farms in Germany by examining three fattening types (slow-growing broilers, layer males, and dual-purpose males) at multiple time points throughout the fattening period.

Methods: The study was conducted on 11 organic poultry farms situated in central Germany. The flock sizes on these farms varied, ranging from 100 to 4,800 birds. The diets provided included a combination of self-mixed rations, complete feed mixes, or pellets. Five farms were dedicated to broiler rearing, four specializing in male layers, and two focused on rearing dual-purpose male birds. Sampling spanned the entire fattening period and was conducted at five distinct time points. Meconium samples were collected on the first day of age from chick transport boxes. Cloacal swab samples were taken at approximately 12 and 30 days of age, two weeks after the chicks were given outdoor access and again at the end of the fattening period. A total of 11 farms were visited, with 50 individuals sampled during each visit. Samples were pooled by combining 10 individual samples into one, and DNA was extracted using a commercial kit. All samples underwent target amplicon sequencing, followed by bioinformatic analysis using QIIME2 [3] and the R programming language. Alpha diversity was assessed using the Shannon diversity index, and statistical significance was determined using the Wilcoxon rank-sum test with Benjamini-Hochberg correction. Beta diversity was calculated using Principal Coordinate Analysis (PCoA) based on Bray-Curtis similarity. The UpSetR package was used to identify taxa similarities. Statistical significance was set at $p<0.05$.

Results: Shannon diversity index showed that meconium contained lower microbial diversity than cloacal samples ($P<0.0001$). As chickens aged, microbial diversity increased, with all cloacal samples containing a significant number of taxa not present in the meconium samples. Samples from chickens with outdoor access contained unique set of taxa, enriching the gut microbiota diversity. PCoA showed distinct clustering by time point when chickens were indoors (12 days and 30 days old). A strong farm effect was observed when chickens had outdoor access. PERMANOVA confirmed significant microbial variation associated with both farm and time factors ($P<0.001$). Microbial composition at the genus level differed significantly between sample types. Enterococcus (55.3%), Escherichia-Shigella (15.3%) and Clostridium sensu stricto 1 (13.7%) dominated the meconium, while cloacal samples were mainly colonized by Lactobacillus (26.2%) and Ligilactobacillus (10.4%). Post-outdoor access, abundance of Bacteroides, which is linked to polysaccharide metabolism, increased to 4.93% and continued to rise over time.

Conclusions: The results indicate that age and environment are key factors in shaping microbiome diversity and composition in chickens within organic farming systems. Current data do not definitively pinpoint specific gut microbiota traits associated with different types of fattening. Ongoing efforts focus on collecting more relevant data and repeated sampling of flocks on the same farms. These efforts aim to elucidate the distinctions between fattening types more clearly.

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48.

Correlation between the potentially human-edible fraction of rations of dairy cows and dry matter intake, energy corrected milk yield and methane emissions

Zusammenhang zwischen dem potenziell human-essbaren Anteil der Rationen von Milchkühen und der Trockenmasseaufnahme, energiekorrigierten Milchleistung und den Methanemissionen

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The decreasing availability of agricultural area in combination with a growing world population leads to an increasing uncertainty of sufficient food supply in future. Consequently feed-food competition (FFC) has to be reduced. Since most of the biomass produced or harvested in agriculture is not human-edible, this biomass should be fed to livestock [1]. Dairy cows, in particular, are able to efficiently convert non-edible biomass into food of animal origin. However, enteric fermentation of ruminants produces methane (CH_4), a greenhouse gas that has an impact on climate. Therefore, the present study investigates the correlation between the potentially human-edible fraction (HEF) of rations of dairy cows on CH_4 emissions, energy corrected milk yield (ECM) and dry matter intake (DMI).

Methods: 48 lactating German Holstein cows were fed a total of 22 different rations varying in components and concentrate feed portion (CFP). The cows were in their first to fifth lactation and live weight ranged between 496 kg and 795 kg. The study consisted of 6 periods conducted over 2 consecutive years, with each period comprising 3 weeks of adaptation and 3 weeks sampling phase. CFP ranged from 20% to 55% of DMI. The rations, which were changed between each period, consisted of varying proportions of different roughages and concentrates. Samples of roughages and concentrates were collected twice a week and once a week, respectively, and were pooled over 3 weeks. All feed samples were analyzed for chemical composition. Energy content of the rations was calculated according to [2]. Feed was offered *ad libitum via* weighing throughs, automatic concentrate feeders (Insentec B.V., Marknesse, The Netherlands) and the Greenfeed system (C-Lock Inc., Rapid City, SD, USA), which was also used to measure CH_4 emissions. Milk samples were taken twice a week at two consecutive milkings, aliquoted and analyzed for fat, protein and lactose. The HEF of the ration was determined on the basis of data from [3]. To cover the range of the HEF, scenarios indicating a "low" (scen_low) and "high" (scen_high) HEF for crude protein of each individual ration component were applied. Scenarios consider dependencies of the HEF on the technology available for processing biomass and the degree of food availability [3]. Data were analyzed with a linear model using RStudio (version 4.3.2) and Spearman's rank correlation coefficient was tested for significance at $p<0.05$.

Results: Daily DMI and ECM ranged from 13.3 to 31.3 kg and from 20.4 to 45.6 kg, respectively. Energy content of the rations varied between 6.4 and 7.4 MJ NEL per kg dry matter. Depending on the scenario, the HEF of crude protein in the rations was between 15.2 and 39.5% (scen_low) and 28.6 and 65.3% (scen_high), respectively. HEF and DMI correlated significantly with $r = 0.21$ (scen_low, $p<0.001$) and $r = 0.45$ (scen_high, $p<0.001$), HEF and ECM correlated with $r = 0.02$ (scen_low, $p=0.75$) and $r = 0.17$ (scen_high, $p<0.01$), respectively. The linear model for HEF and ECM resulted in the following two equations: ECM = 0.05*HEF + 30.48 (scen_low) and ECM = 0.11*HEF + 26.46 (scen_high). Assuming a HEF of the ration of 0 and a lactation length of 300 days, this would result in an annual yield of approximately 8,000 to 9,000 kg ECM without FFC. HEF and daily CH_4 emissions correlated with $r = 0.18$ (scen_low, $p<0.01$) and $r = 0.08$ (scen_high, $p=0.19$), respectively. If CH_4 was related to DMI and ECM, correlation coefficients between HEF and CH_4 per kg DMI were $r = -0.02$ (scen_low, $p=0.75$) and $r = -0.34$ (scen_high, $p<0.001$), and between HEF and CH_4 per kg ECM coefficients were $r = 0.14$ (scen_low, $p<0.05$) and $r = -0.09$ (scen_high, $p=0.13$), respectively.

Conclusions: The correlations between HEF and CH_4 emissions per day, per kg DMI or ECM were not consistently positive or negative indicating that there is no clear trade off between a lower FFC and higher CH_4 emissions. Further research is needed to confirm the linearity between HEF and ECM even at very low HEF.

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49.

How accurate are body weight measurements in suckling calves?

Wie akkurat sind Körpergewichtsmessungen bei nicht abgesetzten Kälbern?

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Body weight measurement is the most crucial criterion for evaluating the sufficient feeding of an animal. Under practical conditions, farmers do not weigh their animals as often as scientists can, but how efficient is weighing in short intervals, and how big is the error we potentially make by weighing a suckling calf? This study explored the possible weight measurement errors from frequently assessed calf weight data. The data were used to calculate the overestimation of average daily gain (ADG) calculations throughout the rearing period of calves to identify the duration after birth when a potential measurement error has negligible influence on data quality.

Methods: Weight data from a feeding study conducted at the TUM research facility Veitshof was used for this analysis. A total of 32 calves of the brown swiss breed were included in the trial. The animals were fed twice daily their dams transition milk and initially colostrum in unrestricted amounts. The calves were housed in calf pens on scales that documented the calf`s weight every ten seconds during their first week of life. This data collection process resulted in a dataset of 65.000 data points for each calf, allowing for high-precision visualization of growth. Due to feces accumulation on the scales, calves were weighed on a separate scale upon exiting the trial, and the error was distributed linearly to each weight measurement. Weight loss curves were calculated from the data, and a best-fit regression (polynomic or linear) was used to describe weight loss between two feeding events. The curves were used to identify time intervals in which the body weight of a calf could be characterized as sober to minimize overestimation of body weight when weighing at any time of the day. Additionally, a polynomial function was used to assess the impact of overestimations on accurate ADG calculations, incorporating a +1 kg error margin for a weight measurement every seven days. The relationship between body weight overestimation and incorrect ADG calculations was analysed for the following periods: days 0-7, 0-14, 0-21, 0-28, 0-35, 0-42, and 0-49.

Results: Growth curves resembled a sawblade-like but linear trend. After feeding, the body weight of a calf reached its maximum, and the minimum was reached immediately before the next feeding. Growth was realized during one feeding period when the following minimum was higher than the last. If the following minimum was lower than the previous, an apparent body weight loss occurred. The weight change between two feeding events was negative and could be described using linear or polynomial functions. When the digestion period was 10 hours (day period), most calves experienced continuous weight loss, which could be accurately represented by a linear function ($R^2 = 0.95$). During the night (14 hours), when the calves exhibited less activity, the weight loss was best described by a 4th-degree polynomial function with an R^2 value of 0.97. For the impact of potential errors on ADG calculations, it was found that a common error of up to 1 kg per weight measurement resulted in less than a 5% error in body weight estimation after day 21. However, earlier ADG calculations were more susceptible to inaccuracies, with deviations of up to ± 14% from the true ADG of the calf.

Conclusions: The experimental setup could create growth curves of calves during their first week of life, which gave insight into the dynamics of weight development. Furthermore, the potential error of body weight measurement was assessed in a scenario resulting in possible overestimations of ADG >5% before 21 days of age and only after 21 days, including 1 kg of measurement error, resulted in less than 5% deviance from the correct ADG calculation. The authors suggest that weighing should be done as often as possible before feeding. However, the potential overestimation of ADG during the first 21 days of a trial (for calves) should be noted and kept in mind when interpreting the data.

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50.

The adaptive character of growth to wear in rabbit incisors

Die Anpassungsfähigkeit des Zahnwachstums an den Abrieb in Kaninchen-Inzisivi

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Throughout evolution, rabbits developed "ever-growing" teeth, which are less vulnerable to their abrasive diet than the brachydont basis. This adaptation, however, predisposes them to tooth overgrowth in a domestic setting. Consequently, many authors recommend feeding rabbits an abrasion-promoting diet to prevent dental disease. The adaptive character of growth to wear noted by others, however, challenges this concept [1]. The present study therefore aimed to better define growth rate of rabbit incisors in response wear.

Methods: The experiment was conducted with 12 female, non-neutered New Zealand White rabbits and consisted of 2 stages. For the first, animals were randomly divided into 2 equal groups and fed either a gruel (no incisor action; 3525 Ranger Kaninchen Spezial, Granovit, 3 parts water to 1 part feed) or a hay-only diet (‚normal' incisor action; perennial ryegrass dominant, stage 5) for 21 days in a cross-over design. Prior to the second stage, all rabbits were fed the hay-only diet for at least one week. Thereafter, the left incisor pair was repeatedly cut with a diamond disk burr, preventing occlusion from recurring to artificially maximize wear. As end point, 8 cuttings per rabbit were taken, lasting up to 54 days. Uncut teeth were marked with a diamond tipped burr to determine wear and growth rates. In cut teeth, changes in length by cutting and regrowth were used. Measurements were transformed to mm/week (unit for the reported values below) and analysed in R v. 4.4.1 using linear mixed models. Significance was set at $p < 0.05$. Additionally, results were combined with data from 7 previous studies and subjected to linear regression analysis to contextualize the relation between wear (independent variable) and growth rate (dependent variable).

Results: No health issues occurred. Diet significantly affected proxies only in the maxillary but not in the mandibular incisors. When fed hay (H) compared to gruel (G), the upper incisors showed increased wear (H: 2.19 ± 0.43 vs. G: 1.01 ± 0.51) and growth (H: 1.95 ± 0.69 vs. G: 1.20 ± 0.38). Values for wear and growth in the lower incisors were respectively H: 1.99 ± 0.45 vs. G: 1.87 ± 0.77 and H: 1.84 ± 0.52 vs. G: 1.53 ± 0.73. Cut teeth showed higher 'wear' (upper: 4.13 ± 0.78; lower: 4.40 ± 0.80) and growth (upper: 3.73 ± 0.44; lower: 4.30 ± 0.57) than uncut ones (wear: 2.29 ± 0.45 and 2.10 ± 0.44; growth: 2.42 ± 0.46 and 2.01 ± 0.50 in the upper and lower jaw). The linear regression analysis ($R^2 = 0.76$) calculated intercepts of 0.63 and 0.38 (95% confidence intervals excluded 0) and slopes of 0.71 and 0.82 for the upper and lower incisors respectively. No data points with zero wear were documented.

Conclusions: This study corroborates in rabbits the adaptive character of incisor growth to wear by dietary or iatrogenic manipulation and that this differs between the upper and lower [1]. The different exposure to wear and pressure could explain this [2]. The regressions indicate a basal growth (intercepts) and an incomplete responsiveness to wear (slopes < 1). However, this and previous work do not consider the uneven circadian distribution of wear and growth [3], resulting in underestimation and overestimation of the slopes and intercepts respectively. As no health issues, nor data points with 0 wear occurred, this study questions the necessity of dietary abrasiveness to prevent dental disease in healthy adult rabbits. It does not contradict the relevance of forage in the rabbit diet for other health aspects.

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51.

Clusters of parameters related to performance and gut physiology in pigs fed two diets of different fibre content under feed choice conditions

Cluster von Parametern der Mast- und Schlachtleistung, sowie der intestinalen Physiologie von Mastschweinen, die mittels Wahlfütterung eine faserarme und eine faserreichere Futteration angeboten bekamen

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Choice feeding allows animals to select between diets with high and low fibre content, potentially leading to optimized nutrient intake and improved performance outcomes. An increase in dietary fibre content, which is typically achieved through the inclusion of industrial by-products in the diet, has been demonstrated to regulate gut physiology and, consequently, contribute to gut health (1). The objective of the study was to gain insight into the potential mode of action by employing statistical clustering techniques in a study where growing pigs were fed two diets of different dietary fibre contents under feed selection conditions.

Methods: The study employed a total of 30 pigs, comprising a mix of barrows and gilts in each pen. The initial body weight of the pigs was 35.9 kg±0.36. The pigs were distributed among three pens according to litter, sex, and body weight. The pigs were provided free access to two dietary treatments, one with a lower dietary fibre content and one with a higher dietary fibre content. To prevent the pigs from becoming habituated to the diet type in the dry-feeding station, the diets in the two feeders were changed on a weekly basis. The pigs were provided with unrestricted access to drinking water. The two diets were formulated to have equal energy contents (ME) and to maintain a constant ratio of energy (ME) to the following nutrients: standardised ileal digestible (SID) Lys, SID Met, SID Thr, SID Trp, Ca, digestible P and Na. All pigs were slaughtered with an average weight of 116.2 kg±0.30, without restricting feeding. A total of 91 parameters, encompassing both fattening and slaughter performance, as well as intestinal physiology-related variables (microbial metabolites and morphometric measurements), were subjected to statistical evaluation. The statistical analyses, which included hierarchical cluster analysis and principal component analysis (PCA), were conducted using the R software.

Results: The mean selection of the lower-fibre diet by the barrows was 45.4±8.4%. The minimum and maximum percentages of feed choice ranged from 32.0% to 61.10%. The mean consumption of the lower-fibre diet by the gilts was 50.4±6.9%. The minimum and maximum choice ranged from 36.65 to 61.45%. Significant differences were observed in the selection of feed between lower and higher fibre diets in barrows ($p<0.05$), whereas no such differences were evident in gilts. The optimal number of clusters was two. The cluster analysis yielded two discrete groups based on growth, feed intake, and metabolic characteristics. Cluster 1 was composed exclusively of barrows, whereas cluster 2 included both barrows (23.8%) and gilts (76.2%). Cluster 1 demonstrated elevated initial body weights, average daily gains, and daily feed intake, yet exhibited less efficient feed conversion compared to cluster 2. Moreover, cluster 1 demonstrated lower concentrations of biogenic amines. Cluster 2 demonstrated superior feed efficiency and lower feed intake. The intestinal microbiota markers demonstrated elevated microbial fermentation, as evidenced by elevated levels of biogenic amines (putrescine, cadaverine, spermidine) in cluster 2. The PCA reveals substantial discrepancies in the metabolic and physiological profiles of the clusters, which are primarily influenced by factors such as feed intake, growth rate, feed conversion, and microbial activity. PCA revealed that PCA1 is predominantly influenced by feed intake and growth rate, indicating that differences in feed consumption and growth are significant in distinguishing the clusters. PCA2 is characterised by the dominance of microbial fermentation products, such as propionate and acetate in the colon. This highlights the crucial role played by microbial activity in differentiating the clusters.

Conclusions: It is probable that the observed differences are influenced by the sex composition within clusters, which affects growth and metabolic responses to diet.

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52.

Influence of feeding a skim milk or whole milk replacer in combination with a soya or pea-based concentrate diet on feed and milk replacer intake and selected blood metabolic parameters in German Holstein calves

Einfluss der Fütterung von Magermilch- oder Vollmilchaustauscher in Kombination mit soja- oder erbsenbasierter Kraftfutterfütterung auf die Tränke- und Kraftfutteraufnahme sowie ausgewählte Stoffwechselparameter im Blut von Deutschen Holstein Kälbern.

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As part of the German government‘s protein crop strategy, this study investigated whether the native legume pea could be a suitable alternative to soya for rearing calves. The aim of this study was to investigate whether feeding a skimmed milk (SM) or whole milk (WM) replacer (MR), and different concentrate feeds (CF) (pea or soya), as well as the combination of MR and CF, influence health and performance with a special focus on MR intake and energy metabolism.

Methods: In a rearing trial, 49 male calves were randomly assigned to four feeding groups. After the colostrum phase, calves received pool herd milk *ad libitum* and were housed in groups at the start of the experimental trial (day 1) at 11 days (11 ± 2 days) of age. Calves were fed either SM MR or WM MR (both NORLAC GmbH, Zeven, Germany) via automatic feeders (Förster Technik GmbH, Engen, Germany). Half of the calves in each MR group were given access to soya-based or pea-based CF administered via automatic feeders (Förster Technik GmbH, Engen, Germany), resulting in the following feeding groups: SM/peas, SM/soya, WM/peas, WM/soya. During the experimental period, calves received hay, water and grass maize silage *ad libitum*. After 28 days, the calves were gradually weaned from MR over a period of 2 weeks. The trial ended on day 42. Daily MR and CF intake were recorded over the whole trial. On experimental day 1, 28 and 42 blood samples were collected from the Vena jugularis externa. Non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), cholesterol, glucose and total protein were measured by using an automatic analysis system (Indiko Plus, Thermo Fisher Scientific, Henningsdorf, Germany). Statistics were calculated using the MIXED procedure of SAS (9.4) including time, MR, CF, and their interactions as fixed factors. For all blood parameters, the baseline sample was considered as a covariate.

Results: Within four days, the MR intake increased to the maximum of 1.44 kg/d and calf and remained at this level until weaning, with no differences between groups. During the weaning, all calves, regardless of the group, consumed the maximum amount of MR that was offered. In the whole period between day 1 and the start of the weaning on day 28, all pea-fed calves consumed a total of 1.5 kg of concentrate, while all soya-fed calves consumed the 3.9-fold (5.8 kg). Regardless of MR, concentrate intake during the weaning period increased substantially in soya-fed calves to 1.5 kg/d and calf, whereas the pea-fed group consumed significantly less concentrate (0.18 kg/d/calf) until the end of weaning.All blood parameters varied significantly with time ($p_{time} < 0.001$), except for NEFA. Pre-weaning, blood glucose, BHB and total protein remained constant regardless of CF and MR. During weaning, blood glucose decreased while BHB and total protein increased in all calves until the end of the trial. Depending on MR, there was a significant effect on the cholesterol concentrations on experimental day 28 and 42 ($p_{time*MR} < 0.001$). While the cholesterol concentration of SM doubled in the period between experimental day 1 (2.1 mmol/l) and day 28 (4.17 mmol/l) and remained constant during weaning, the concentration in WM increased slowly over the entire measurement period. Blood NEFA differed significantly between MRs ($p_{MR} = 0.022$). In the SM group, the NEFA concentration was on average 0.044 µmol/l lower than in the WM group.

Conclusions: In conclusion, dietary changes during weaning affected calf energy metabolism parameters regardless of calf feeding group (CF, MR). Nevertheless, MR was shown to have a particular effect on lipid metabolism parameters. However, data from the subsequent fattening trial on the same animals need to be analysed to investigate the effect of MR and CF on health and performance in the long term.

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53.

Influence of feeding a milk replacer with whole milk or skimmed milk combined with a concentrate of soy or peas on growth of male Holstein calves

Einfluss der Fütterung mit einem Magermilch- oder Vollmilch-Milchaustauscher in Kombination mit einem Kraftfutter aus Soja oder Erbse auf das Wachstum von männlichen Holstein-Kälbern

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Reducing soy imports could help prevent deforestation and reduce carbon footprints. Regionally produced peas could contribute to this strategy. Thus, the aim of this study was to evaluate the effects of early feeding of different protein source in a concentrate feed (CF, soy vs. peas) in combination with different milk replacers (MR, whole milk (WM) vs. skimmed milk (SM)) on the growth of male German Holstein calves. We hypothesised that there will be no difference in the growth of male Holstein calves between a pea-based and a soy-based concentrate, but that a whole milk based MR will result in better growth performance than a skimmed milk based MR.

Methods: In a rearing trial, male calves (N=49) were randomly assigned to four feeding groups, with calves from primiparous cows (n=15) evenly distributed among the groups. At the start of the trial, calves (age 11 ± 2 days) were housed in groups and fed a maximum of 9 L/day either a SM MR or WM MR (both NORLAC GmbH, Zeven, Germany) via automatic feeders (Förster Technik GmbH, Engen, Germany). Half of the calves in each MR group were given access to up to 2 kg/day of either soy-based or pea-based CF administered via automatic feeders (Förster Technik GmbH, Engen, Germany), resulting in the following feeding groups: SM/peas, SM/soy, WM/peas, WM/soy. After 28 days, the calves were gradually weaned by reducing the MR from 1.44 kg/day to 0.32 kg/day over two weeks. Throughout the experimental period, calves received hay, water and grass and maize silage *ad libitum*. The experiment lasted from day 1 to 42, with a baseline sample taken 48 hours after birth. Morphometric data such as body weight, withers height, hip height, heart girth, back length and body length were recorded weekly and average daily gain of body weight (ADG) was calculated. Ultrasonic data of back fat thickness [1] and rip fat thickness [2] were determined on experimental day 28 and 42 with a portable ultrasound device (Esaote Biomedica Deutschland GmbH, Köln, Germany). Statistical analysis was performed using the PROC MIXED procedure in SAS (V 9.4., SAS Institute Inc., Cary, NC, USA) with time (T), MR, CF and mother's parity (P) as fixed factors, and interactions between T, MR and CF and an adjusted Tukey-test as a post-hoc procedure. In addition, the baseline sample was considered as a covariate for body weight, withers height, hip height, heart girth, back length and body length.

Results: The calves started the trial with an average body weight of 43.15 ± 6.36 kg and showed constant growth in all morphometric variables over time ($p<0.05$), except for back and rip fat thickness. There were no significant interactions between CF and time, and between MR and CF. Similarly, no significant interactions between the MR and CF over time were found. A significant interaction between MR and time was observed for body weight ($p_{MR*T}=0.039$), hip height ($p_{MR*T}=0.010$) and ADG ($p_{MR*T}=0.025$), with calves from the WM group having a higher hip height and a higher body weight during the weaning period than calves from the SM-fed group. ADG tended to be higher in WM than in SM groups on day 21 ($p=0.058$). Heart girth ($p_P=0.003$), back length ($p_P=0.037$), body length ($p_P<=0.030$) and rip fat thickness ($pP=0.036$) were statistically significant influenced by maternal parity, resulting in lower heart girth, back length and body length in calves from primiparous cows, but higher values for rip fat thickness.

Conclusions: The hypothesis can be confirmed in that there is no difference in growth of male Holstein calves between a pea-based CF and a soy-based concentrate feed. The hypothesis that a WM-based MR results in better growth performance compared to a SM-based MR cannot yet be answered conclusively, as the effects were only observed at the end of the weaning trial. After this initial observation of the weaning phase, the bulls are subjected to further trials during the subsequent rearing and fattening phase.

Acknowledgement: This study was funded by the Federal Office for Agriculture and Food.

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54.

Estimation of the milk intake of cow-bound calves in the first week of life based on the growth rate

Schätzung der Milchaufnahme kuhgebundener Kälber in der ersten Lebenswoche anhand der Wachstumsrate

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Sufficient milk intake is crucial for the health and development of calves at a juvenile age. Conventionally separated (CS) calves are fed with a teat bucket twice daily. In cow-bound (CB) rearing systems, which are becoming increasingly important in agricultural practice, calves can suckle milk from the udder in unlimited amounts and several times during the day. This study aimed to estimate the milk uptake of CB calves based on the data from CS calves.

Methods: The study was conducted on the TUM experimental farm Veitshof with brown swiss breed‘s calves (n = 20). CS calves (n = 10) were housed in calf hutches immediately after birth (< 1 h) and were fed colostrum and transit milk from their mother in unlimited amounts twice daily until drinking was terminated for at least one minute. Provided and the remaining milk was weighed, and milk consumption was calculated. CB calves (n = 10) were allowed to suckle all day in the calving pen until their 7th day of life. Additionally, cows were milked twice daily. CS and CB calves were weighed immediately after birth and on the morning of day seven after at least three hours milk withdrawal. The literature shows that twice-daily feeding results in differences in body composition compared to more frequent feeding, but not in nutrient or energy balance, heat production or weight gain. All data were transferred from recording sheets to an Excel file. The statistical analysis was performed with RStudio 4.4.2 (R Core Team, Vienna, Austria). A multiple linear regression was carried out to explain the weight of CS calves by milk consumed, sex, birth weight, weight on day seven, fecal score, and the temperature-humidity index. The equation was used to estimate the milk intake of CB calves.

Results: CS calves showed comparable ($P > 0.26$) high weight gains (9.56 ± 1.89 kg/week) to CB calves (10.56 ± 1.95 kg/week). Values of weight gain from birth include partly an effect of the filling of the digestive tract. The equation was suitable for estimating milk intake in CS calves ($R^2 = 0.98$). The milk intake (53.75 ± 8.29 L/week) of the CS calves was still lower ($P < 0.02$) compared to the estimated milk intake of CB calves (64.95 ± 7.33 L/week).

Conclusions: Estimated unlimited milk intake in CB calves is feasible, as they drink more than 10 times per day and more volume compared to twice daily unlimited of CS calves. The higher activity of CB calves might explain the higher milk intake with lacking body weight gain difference.

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55.

Influence of dietary fat sources on performance, body composition, and fatty acid profiles in *Hermetia illucens* larvae

Einfluss diätetischer Fettquellen auf Leistung, Körperzusammensetzung und Fettsäureprofile von Hermetia illucens-Larven

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Black soldier flylarvae (BSFL, *Hermetia illucens*) are increasingly recognized as an alternative source of proteins and lipids for food, feed, and biotechnological applications. Their lipid profile is influenced by diet, which in turn affects their potential as a fat source in animal feed [1]. The type and source of dietary fats modulate both the quantity and quality of stored fatty acids, including the balance of saturated, monounsaturated, and polyunsaturated fats [2]. Understanding the relationship between dietary fats and larval lipid metabolism is crucial for optimizing their fatty acid composition. This study aims to evaluate the effect of different dietary fatty acid profiles on the energy, fat, nitrogen, ash, and fatty acid concentration in BSFL.

Methods: A total of 420,000 6-day-old BSFL (7.0 mg ± 0.4; madebymade GmbH, Pegau, Germany) were reared in a controlled climate chamber (28°C, 55% rH) and randomly assigned to five dietary treatments. Each isonitrogenous diet contained 5% fat (DM basis) from different fat sources: beef tallow (BT), olive oil (OO), sunflower oil (SO), linseed oil (LO), and a basal diet (BD) as a control group (14,000 BSFL per rearing box; n = 6/group; N = 30). Diets were offered *ad libitum* (0.2g DM/larvae). After 8 days of rearing, the BSFL biomass (rearing box) was separated from the feeding substrate, washed, dried, weighed, and frozen (-18°C). Feed and BSFL were analyzed for gross energy (GE; bomb calorimetry), fat, nitrogen and FA content and profile. To determine the FA profile, the fat was extracted with heptane from freeze-dried and pulverized samples according using Soxhlet extraction. The extracted triglycerides were trans-esterified to fatty acid methyl esters (FAME). The FAME mixture was analyzed and identified with a gas chromatograph. Data were statistically analyzed using ANOVA with the Tukey HSD, $P \leq 0.05$ (IBM SPSS Statistics, Version 29). Results are presented as mean ± SEM.

Results: The diet affected BSFL performance. BSFL fed BD showed the lowest average daily weight gain (201.2±6.4 g/d/box; P=0.024) and survival rate (87.2±1.6 %; P<0.001), while BSFL fed BT had the highest (245.7±4.7g/d/box; 96.3±2.2 %). Regardless of diet, the fat composition of the BSFL was characterized mainly by lauric acid (LA) and other saturated fatty acids (SFA), which were synthesized de novo, as neither LA was present in any of the five feed substrates. BSFL reared on SO and LO substrates showed the lowest level of SFA (SO:46.4±3.1 and LO:48.1 ±3.7 g/100 g of total lipids, respectively, P<0.001), while the animal-based fat in diet (BT) increased the level of SFA (62.3±2.5 g/100 g of total lipids). Larvae fed SO and LO had the highest (SO:23.0±0.2 and LO:24.2±0.7 g/100 g of total lipids; P=0.004) levels of polyunsaturated fatty acids (PUFA) and larvae fed BT and OO had the lowest (BT:11.2±1.4 and OO:15.3±0.9 g/100 g of total lipids). However, there were no significant differences in GE, fat and nitrogen concentration among larvae fed diets with added fat. Ash content was highest in BSFL fed BD and lowest for BSFL fed BT (9.1±0.3 and 6.2±0.5 g/kg DM; P<0.001)

Conclusions: BSFL in this study showed plasticity in their fatty acid (FA) profile, which can be modulated by altering dietary fat sources. This adaptability suggests the potential to influence the nutritional properties of BSFL as feed. However, these changes reflect the larvae‘s ability to adjust to varying diets, not necessarily their precise nutritional requirements. Higher levels of fatty acids from endogenous synthesis were found in BSFL fed diets with added fat, particularly those rich in polyunsaturated fatty acids (PUFA), possibly due to lipogenesis inhibition by the added fat [2].

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56.

Impact of reduced nitrogen and/ or phosphorus supply on the growth hormone receptor-IGF1 axis in small ruminants

Einfluss einer reduzierten Protein- und/ oder Phosphatversorgung auf die Wachstumshormonrezeptor-IGF1-Achse bei kleinen Wiederkäuern

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The diet of farm animals plays a significant role in environmental sustainability, influencing both resource consumption and the excretion of metabolic byproducts. Understanding the physiological mechanisms behind animal metabolism is essential for optimizing feeding strategies. Ruminants, in particular, serve as a suitable target for reducing nitrogen (N) and phosphorus (P) levels in feed due to their ability to recycle P and utilize N through forestomach microbes. However, simultaneously lowering N and P poses significant challenges, as it may disrupt various metabolic pathways. Previous research has demonstrated that reducing N intake disrupts the somatotropic axis in young goats by impairing the growth hormone receptor (GHR)-insulin-like growth factor 1 (IGF1) pathway, leading to lower IGF1 levels (1). This reduction is linked to decreased GHR expression and reduced insulin levels. Similarly, in monogastric animals, decreased P intake has been associated with reduced insulin levels (2). Therefore, it was hypothesized that, like N reduction, reduced P supply could also impair the somatotropic axis, possibly through a similar mechanism. This study aimed to investigate the effects of reduced N and P intake on the GHR-IGF1 axis in young goats to better understand the physiological consequences of these dietary modifications.

Methods: Twenty-eight male, growing goats of the Bunte Deutsche Edelziege breed were assigned to four groups, each consisting of seven animals: a control group (n = 7; 14% N and 0.45% P), an N-reduced group (n = 7; 6.5% N and 0.45% P), a P-reduced group (n = 7; 14% N and 0.10% P), and a group with both reduced N and P (n = 7; 6.5% N and 0.10% P). The feeding trial lasted for six weeks, after which the goats were slaughtered, and tissue and blood samples were collected. Plasma concentrations of urea, total calcium, and inorganic phosphate were measured using standard colorimetric methods. Additionally, plasma growth hormone (GH), serum IGF1, and plasma insulin levels were analyzed at the Clinic for Cattle, Laboratory of Endocrinology, University of Veterinary Medicine Hannover, Germany. Liver samples were collected post-slaughter to assess the expression of GHR and IGF1 pathway components via qPCR. Data were analyzed using two-way ANOVA followed by Tukey's multiple comparison test, with statistical significance set at $p < 0.05$.

Results: Goats on the P-reduced diet exhibited a significant reduction in blood IGF1 levels ($p = <0.0001$), similar to the reduction observed in goats on the N-reduced diet. However, GH and insulin concentrations remained unaffected by either dietary modification. Despite the dietary changes, GHR mRNA levels were unchanged. Under N reduction, the expression of JAK2, STAT1 ($p = <0.05$), and STAT5b ($p = <0.05$) was elevated, just like SOCS1, SOCS2 ($p = <0.005$), and SOCS3 ($p = <0.05$) mRNA levels increased. In contrast, P reduction did not affect components of the GH-IGF1 axis, but it did result in reduced IGF1 mRNA expression ($p = <0.05$).

Conclusions: These findings suggest that the reduction in IGF1 is likely mediated by alternative mechanisms beyond insulin-regulated GHR modulation. The increased expression of JAK2, STAT1, and STAT5b during N restriction points to the involvement of intracellular signaling pathways in the downregulation of IGF1 production. Additionally, the reduction in IGF1 mRNA expression observed with P restriction, despite the lack of disruption to other components of the GH-IGF1 axis, highlights the complexity of nutrient-driven regulatory mechanisms. Further research is needed to explore the precise pathways responsible for the observed effects, which could provide insights into optimizing feeding strategies for improved animal health and environmental sustainability.

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57.

Effect of feeding insect meal on the expression of genes involved in protein synthesis and degradation in breast muscle of broilers

Wirkung der Fütterung von Insektenmehl auf die Expression von Genen der Proteinsynthese und des Proteinabbaus im Brustmuskel von Broilern

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Alternative protein sources are increasingly needed for animal production to face the challenge of worldwide increasing demand for products of animal origin and the increasing shortage of natural resources, such as arable land. In a recent study, we observed that meal from larvae of the black soldier fly (*Hermetia illucens*, HI) can be used as a source of protein at levels of 150 g/kg diet at the expense of soybean meal in broiler diets without adverse effects on performance or metabolism. Indeed, in this study broilers fed HI larvae meal exhibited higher breast muscle weights at identical concentrations of crude protein and digestible amino acids than broilers of the control group, although body weights were not different between the groups [1]. That observation led us to the hypothesis that feeding diets with HI meal resulted in a change in muscle protein turnover. For this end, we determined the expression of genes involved in the growth hormone-insulin-like growth factor (IGF)-1-axis in the liver and genes involved in pathways of protein synthesis and protein degradation, including the mammalian target of rapamycin (mTOR) pathway, myogenesis, the ubiquitin proteasome system (UPS), autophagy and the control nonderepressible 2 (GCN2)/eukaryotic translation initiation factor 2A (eIF2a) pathway in breast muscle.

Methods: 72 male, 1-day-old Cobb 500 broilers were randomly assigned to three groups and fed three different nutritionally adequate diets, which contained either 0 (HI-0), 75 (HI-75) or 150 (HI-150) g HI larvae meal per kg diet, in a three-phase feeding system for 35 days. HI meal was added to the diets at the expense of soybean meal at an isonitrogenous base. Concentrations of digestible indispensable amino acids were adjusted to identical concentrations within the three types of diet. After killing, breast muscle samples were taken. Relative mRNA concentrations of genes were determined by qPCR. Normally and not-normally distributed data were analyzed by one-way ANOVA and Kruskal-Wallis test, respectively, followed by Tukey´s post-hoc test for significant F values. Effects were considered significant if $P<0.05$.

Results: Breast muscle weights of the three groups of broilers (g, means ± SD) were: HI-0, 582 ± 71^{b}; HI-75, 639 ± 102^{a,b}; HI-150, 661 ± 80^{a} ($P<0.05$) [1]. Relative mRNA concentrations of growth hormone receptor (GHR), IGF-1 receptor (IGF1R) and IGF-binding protein 2 (IGFBP2) in breast muscle were not different between the three groups. Relative mRNA concentration of IGF-1 in breast muscle was lower in groups HI-75 and HI-150 than in group HI-0 ($P<0.05$). Relative mRNA concentrations of MTOR and of protein S6 kinase (S6K1) and eukaryotic initiation factor 4E binding protein 1 (4EBP1), two genes involved in the anabolic mTOR pathway, did not differ between the three groups. Relative mRNA concentrations of two genes involved in myogenesis [class I myosin (MyoD) and myogenin (MyoG)], were not different between the three groups of broilers. Relative mRNA concentrations of two genes involved in the UPS [F-box only protein 32 (FBXO32), muscle RING finger 1 (MuRF1)] and forkhead box protein O1 (FOXO1) transcription factor, which induces protein degradation, were not different between the three groups. Relative mRNA concentrations of genes involved in the GCN2 pathway [sequestosome 1 (SQSTM1)] and autophagy [autophagy related 5 (ATG5), autophagy related 6b (ATG6b), autophagy related 9 (ATG9)] were not different between the three groups of broilers.

Conclusions: The present findings suggest that a modulation of the protein anabolic or protein catabolic pathways considered in this study is unlikely to explain the increased relative breast muscle weight in broilers fed diets containing HI larvae meal as a source of protein. However, it cannot be excluded that feeding insect meal affected other less prominent pathways which have not been considered in this study but which are also involved in the regulation of protein turnover,

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58.

Application of probiotic lactic acid bacteria as a feed additive in broilers

Verwendung von probiotischen Milchsäurebakterien als Futterzusatz für Masthähnchen

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Probiotics are defined as live microorganisms which, when administered in adequate quantities, confer health benefits on the host. The main benefits promoted for the use of these microorganisms as feed additives are associated with modulation of the balance and activity of the intestinal microbiota, resulting in improved health and immunity of the host's intestinal mucosa. The use of probiotics in the prevention and treatment of bacterial infections in animals intended for human consumption is considered an effective alternative to the use of antibiotics. As natural inhabitants of the intestinal microflora, Lactic Acid Bacteria (LAB) exert a positive influence on the intestinal health of the host by improving the nutritional and immunological status of the host. The use of probiotics LAB reduces the risk of antibiotic resistance and improves the sustainability of poultry production. The hypothesis is that the LAB strains selected for use as probiotics will be able to improve animal response.

Methods: *in vitro* assays were performed to identified physical-chemical properties (hetero/homofermentative; gastrointestinal tolerance; adherence to intestinal cells; vitamin production; virulence factors; antibiotic resistance; bacteriocin production) of each LAB and their ability to coexist in the same environment (coexistence text) to determine the existence or absence of inhibition between the strains. The *in vivo* study was performed by the administration of the additive in the feed every 3 days, due to the established feeding routine at the experimental station, at a concentration of 5.0 × 108 CFU/kg of feed for one-day broilers. It was carried out for 35 days with 6 boxes containing 15 animals/box for each treatment: negative control (absence of additive and capsule), positive control (presence of empty capsule), non-encapsulated probiotic additive and encapsulated probiotic additive. The capsule was made of alginate containing the prebiotic mannan. The results, expressed as mean values ± standard deviations, were subjected to one-way analysis of variance (ANOVA) and compared using Tukey's post-hoc test with a significance level (P) < 0.05.

Results: According to the negative *in vitro* results on the virulence factors, the additive was formulated with four different strains of Pediococcus pentosaceus which showed no inhibition to each other. They had low rate of antibiotics resistance, good tolerance to the conditions of the gastrointestinal tract, good ability to adhere to intestinal cells and good inhibition effect against the growth of Salmonella spp. Considering feed intake (FI) in 28 and 35 days of experiment, the animals that received the probiotic additive (encapsulated or not) showed lower values (FI = 1943 g and 1892 g, respectively) compared to the control (FI = 2289 g), indicating greater satiety for the animals that received the probiotic additive. Even though the data did not show any statistical difference (P = 0.07), the results for the days 28th and 35 th indicate better performance for the animals that received the probiotic additive. Differences in feed conversion ratio (FCR) were statistically significant for the days 28th (P = 0.02) and 35th (P = 0.01), with better results for the animals that received the encapsulated (FCR = 1.04 and 1.16, respectively) and non-encapsulated (FCR = 1.02 and 1.09, respectively) additive in relation to the control (FCR = 1.29 and 1.46, respectively). While the animals that received the encapsulated and non-encapsulated additive ate less feed from the 28th day onwards, they showed greater body weight and weight gain than the animals in the control group (absence of additive) (P ≤ 0.05), an important correlation indicating a possible trend towards a probiotic effect of the additive administered.

Conclusions: Therefore, with the data obtained so far, we can speculate that this additive has a high chance of being proven as a probiotic additive, which will be proven with the conclusion of the ongoing analysis.

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59.

Evaluation of diclazuril, robenidine and oregano oil as feed additives for the prevention of coccidiodis in growing rabbits artificially inoculated with *Eimeria spp*.

Evaluierung von Diclazuril, Robenidin und Oregano-Öl als Futterzusätze zur Kokzidioseprävention bei experimentell inokulierten wachsenden Kaninchen

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Interest in natural alternatives to conventional coccidiostats for preventing coccidiosis in fattening rabbits is growing. First results obtained in a field study indicated that in the absence of the most pathogenic Eimeria species, economic rabbit rearing and fattening is achievable without the use of coccidiostats [1]. The present study aimed to compare possible anticoccidial effects of oregano oil to diclazuril and robenidine in growing rabbits under experimental inoculation with the most common and one of the most pathogenic *Eimeria (E.)* species (*E. flavescens*).

Methods: 48 five-week-old SPF rabbits (New Zealand White) were allocated to one of 16 groups and fed four different diets (four replicates per diet; three rabbits per replicate and cage). In addition to an *ad libitum* offer of hay, the control group (C) received a pelleted compound feed based on alfalfa, wheat bran, and oat hull meal as basal diet. Diclazuril (1 mg/kg; D), or robenidine (66 mg/kg; R) or oregano oil (*Origanum vulgare*, 75 mg/kg; O) were added. Experimental Eimeria inoculation was performed 10 days after the rabbits arrived. Each rabbit received a dose of 1,300 oocysts containing *E. media, E. magna, E. flavescens, E. coecicola*, and *E. perforans*. Oocyst excretion was monitored and *Eimeria* species were identified 4-14, 17, 21, 24 and 28 days *post infectionem* (dpi). Excreted oocysts were determined as absolute numbers (per rabbit and day) and the reproduction of each *Eimeria* species was calculated. Performance data (compound feed and water intake, growth rate and feed conversion rate) and faecal quality were determined. The hay intake was measured on faeces collection days. At day 32 pi, samples were obtained from contents of the jejunum and caecum of each rabbit (n = 12 per group) for microbiota analyses (16S rRNA gene sequencing). Depending on the data distribution, parametric or nonparametric tests were used for group comparisons. Time comparisons were done with a grouped one-way ANOVA for paired samples in SAS Enterprise Guide (version7.1). Microbiota composition of samples were assessed for variation in relation to the diet by a permutational multivariate analysis of variance (PERMANOVA) using the Bray-Curtis distance in R (version 4.4.0).

Results: Oocyst excretion varied at 4-14 dpi within groups according to the different prepatent periods of the *Eimeria* species. Mean oocyst excretion increased in all groups, peaked on 7 and 8 dpi and decreased thereafter. The reproduction of the medium and high grade *Eimeria* was in the order C, D, R and O as follows (in 10^6 oocysts): E. magna: 186 ± 18.1, 164 ± 24.5, 182 ± 22.3, and 196 ± 10.9 (p = 0.199); *E. media*: 132 ± 8.92, 120 ± 20.7, 120 ± 3.79, and 142 ± 9.21 (p = 0.073); E. flavescens: 33.1 ± 11.3, 32.7 ± 12.2, 31.6 ± 9.18, 35.3 ± 7.47 (p = 0.964). In all groups, compound feed intake was reduced from 3-8 dpi, whereas hay intake remained relatively constant. Mean daily compound feed intake amounted in g dry matter: 112 ± 35.2 in C, 111 ± 33.6 in D, 114 ± 35.7 in R, and 111 ± 35.0 in O, while mean daily hay intake was in C: 11.8 ± 3.64, D: 13.1 ± 3.27, R: 13.5 ± 2.94, and O: 13.9 ± 4.04, representing about 10% of dry matter intake. Neither oocyst excretion nor any of the performance parameters or faecal quality differed significantly between the four groups. Microbiota richness and diversity did not differ between the groups, but PERMANOVA revealed significant differences in microbiota composition in the jejunum between C and O (p = 0.027) and in the caecum between O and D (p = 0.041), and O and R (p = 0.001).

Conclusions: Neither diclazuril and robenidine nor oregano oil were superior to non-supplemented diet in terms of oocyst multiplication or zootechnical parameters. The diversity and composition of the intestinal microbiota of the rabbits that received the coccidiostats did not differ from rabbits offered a non-supplemented diet. Oregano oil supplementation appears to affect caecal microbiota composition differently from coccidiostats.

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60.

Effects of a phytogenic feed additive (PFA) composed of Cinnamaldehyde, Carvacrol, 1,8-Cineole, Capsaicin, Garlic Extract and Fenugreek on performance and post weaning diarrhea indices in weaned piglets compared with zinc oxide

Wirkungen eines phytogenen Futterzusatzes (PFA) bestehend aus Zimtaldehyd, Carvacrol, 1,8-Cineol, Capsaicin, Knoblauch-Extrakt und Bockshornklee auf Leistungsparameter sowie Indikatoren für Absetzdurchfälle bei Absetzferkeln im Vergleich zu Zinkoxid

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Since June 2020 dietary zinc oxide (ZnO) at pharmacological doses as growth promoter and as a preventing agent against post weaning diarrhea (PWD) in piglets has been banned by law in the European Union. Due to this fact research in alternative solutions has gained in importance. Besides organic acids and probiotics, in particular phytogenic feed additives (PFA) are promising candidates for ZnO replacement. Whereas essential oils have been demonstrated to reduce the virulence of enterotoxic *E. coli* bacteria by Quorum Sensing Inhibition, Fenugreek can prevent intestinal biofilm formation of pathogenic bacteria due to its high Galactomannan content. Pungents, like Capsaicin and Garlic help to improve nutrient digestion and absorption. Consequently, the aim of the current study was to investigate the efficacy of a phytogenic feed additive composed of 20% matrix encapsulated essential oils (Cinnamaldehyde, Carvacrol, 1,8 Cineole), 0,2% Capsaicin, 16% Garlic Extract and 63,8% Fenugreek Powder, compared to ZnO, on performance and diarrhea indices in weaned piglets under practical conditions. The single compounds of the PFA have been selected based on their Mode of Action.

Methods: At 24 days of age 240 weaned piglets (120 female / 120 male) from Asmussen Agro GmbH, Jessen, Elster (Germany) with an average body weight of 6.90±0.79 kg were allocated to 3 experimental treatments: (1) Negative Control (NC = no additive), (2) Positive Control (PC = 3000 mg/kg diet ZnO) and (3) PFA (500 mg/kg diet). Each treatment consisted of 8 repetitions with 10 piglets each. The piglets were fed commercial diets without (NC) or with (PC, PFA) the additives, respectively. The entire study lasted for 42 days and consisted of a starter phase (d 25 to d 38 = 14 days) and a grower phase (d 39 to d 66 = 28 days). The study was accompanied by the Institute of Animal Nutrition, Free University, Berlin. During the experiment, performance, diarrhea incidence and feces consistency were controlled at days 7, 14, 28 and 42 on trial. Results are presented as Means ± Standard Deviation. The study design followed a random complete block design, with the pen as the experimental unit for statistical evaluation of recorded parameters. All performance and diarrhea parameters were analysed by a one-way ANOVA using the software package SPSS (IBM SPSS Version 21).

Results: According to the trial design, the initial mean body weight did not differ between the experimental goups (6.90±0.79 kg). After the 14 days starter period, PC piglets showed the highest body weight (12.18±0.93 kg), followed by PFA piglets (11.73±1.11 kg) and the NC animals (11.24±1.01 kg). With (0.31±0.031 kg/d) the achieved daily weight gain in the NC group differed highly significant ($p<0.001$) from PC piglets (0.37±0.014 kg/d) and significantly ($p<0.05$) from PFA piglets (0.35±0.014 kg/d). High significant differences ($p<0.01$) were achieved between FCR in group NC (1.177±0.062 kg/kg) compared to groups PC (1.077±0.030) and PFA (1.095±0.037) group. The observed effects continued in the 28d grower period. With 29.19±1.29 kg group PC achieved the highest final body weight compared to groups PFA (27.50±1.37kg) and NC (26.34±1.54) at significant differences in FCR ($p<0.01$ and $p<0.05$) between PC piglets (1.459±0.047) and PFA piglets (1.489±0.048) compared to NC animals (1.530±0.040), respectively. Total PWD incidence was 5% in group NC, 0% in group PC and 1,25% in the PFA group. Seven days of feeding ZnO (0.32±0.15 kg) or the PFA (0.30±0.28 kg) resulted in highly significant ($p<0.001$) firmer feces compared to group NC (0.86 kg±0.37). This effect was conserved during the entire experimental period ($p<0.01$).

Conclusions: The present study has demonstrated that PFA can play an important role as ZnO replacers. It must be considered that in the current study deliberately an extremely high ZnO dose has been chosen, to show genuine effects of the PFA.

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61.

Impact of inactivated brewer's yeast, autolysed or not, and a probiotic strain of *Saccharomyces cerevisiae* added to a high starch diet on gas production and microbial fermentation products *in vitro* (Batch culture)

Einfluss der Zugabe inaktivierter Bierhefe, autolysiert oder nicht, und eines probiotischen Stammes von Saccharomyces cerevisiae zu einer stärkereichen Ration auf Gasbildung und mikrobielle Fermentationsprodukte in vitro (Batch-Kultur)

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Saccharomyces cerevisiae plays a notable role in horse feeding, either as inactive brewer's yeast (BY) with prebiotic properties [1] or viable as an approved probiotic (LY). Although fed in different quantities and basing on varying assumed modes of action, they are used for similar purposes (e.g. avoidance of free faecal liquids). However, yeast cell walls are very resistant and autolysis might thus increase the accessibility of yeast cell components for microbial fermentation. The recent *in vitro* study was conducted as proof of principle to compare gas yield and microbial fermentation products from BY vs autolysed BY (aBY) and contrast this with LY effects when added to a high starch diet during inoculation with equine faeces.

Methods: In a batch culture system (ANKOM RF Gas Production System) with buffer adapted to conditions in the equine hindgut [2]either 0,0225 g of SC(MUCL 39885) or BY or aBY were added to a core diet consisting of 0,155 g meadow hay and 0,045 g wheat starch of lab purity were the core diet only served as control (CO). The inoculum was prepared from mixed faeces from three adult healthy horses. In each of entirely five runs, three shaking water baths were equipped with two fermenters per diet each and inoculated for 48 hours together with two blind fermenters each without inoculum only. During incubation, the gas release from each vessel was detected every 10 sec. Finally, vessels were opened, fluids sampled, and investigated for pH, ammonia (Conway method), short chain fatty acids (SCFA), lactate and alcohols (ethanol, 1-propanol, 1,2-propandiol; HPLC). The gas production curve was modulated via the Gompertz function, LSMeans ± standard errors were estimated for pH and microbial fermentation products and diet effects investigated using the procedure MIXED with $P < 0.05$ considered significant (SAS 9.4).

Results: The estimatedtotalgas production showed the following gradation: CO > LY > BY > aBY ($P < 0.05$). Time to achieve one third of the entirely produced gas was shortest with CO < LY = BY < aBY ($P < 0.05$), and to yield between one third and 70% of the total gas production was shortest with aBY ($P < 0.05$). The final pH varied between 6.7 – 6.8. CO vessels had the highest pH and lowest contents of total SCFA, acetate, n-butyrate, iso- and n-valeriate and ammonia ($P < 0.05$). Total SCFA concentration was highest with BY and aBY ($P < 0.05$). Both, contents and molar percentages of n-butyrate, iso- and n-valeriate were highest with aBY ($P < 0.05$). Lactate and alcohols were below the limit of detection.

Conclusions: Total SCFA reveal highest fermentation with BY, autolysed or not. Autolysis of BY however particularly elevated microbial production of n-butyrate, which can be assessed as prebiotic effect [3]. This and the high increase in gas production during a developed stage of inoculation indicate a better accessibility of yeast cell components through autolysis.

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62.

Performance and nutrient digestibility in weaned piglets fed with increasing levels of dietary phytase

Leistungsparameter und Nährstoffverdaulichkeiten bei Absatzferkeln mit höheren Phytase-Gehalten in der Ration

Schulze Holthausen J., Männer K., Ebersbach L., Fickler A., Ader P., *Zentek J. – Berlin / Ludwigshafen

In plant seeds used as feed, 60–80% of the phosphorus (P) is bound to phytate, making it unavailable to monogastric animals like swine, as they lack the necessary intrinsic enzyme to break it down. This study aimed to evaluate the impact of increasing levels of phytase on growth performance and nutrient digestibility in weaned piglets.

Methods: Sixty-four piglets were weaned at day 25, transferred to pens with 2 piglets per pen (1 male/1 female) and received a standard creep feed for the following four days. At day 29 of age, piglets were randomly assigned to one of four dietary treatments, comprising a control diet (T1) and three further control-based diets supplemented with a hybrid bacterial 6-phytase (Natuphos® E 5000 G, BASF SE) at levels finally analysed for 748 FTU/kg (T2), 2185 FTU/kg (T3), and 3930 FTU/kg (T4). The diets were formulated to meet the nutritional requirements of piglets [1], but digestible P was 0.26 g/kg feed. Main ingredients of the basal diet were maize (62.8 %), soybean (44) (11.8 %), sunflower (11.3 %), and rapeseed meals (8.00 %). Celite was included as indigestible marker. Piglets were housed under controlled conditions, with *ad libitum* access to feed and water throughout the 21-day lasting experimental period. Body weight, feed intake, and feed-to-gain ratio were recorded weekly. At the end of the trial, apparent praecaecal digestibility of crude protein, fat, calcium (Ca) and P were determined. Ileum digesta samples were collected from euthanized male piglets, in addition, faecal samples were collected for determination of apparent total-tract digestibility of Ca and P. All data were analyzed using analysis of variance (ANOVA) with treatment as the fixed effect and the pen as the experimental unit. Multiple comparisons between treatment groups were performed using Tukey's test, and significant differences were declared at $P \leq 0.05$.

Results: Piglets receiving phytase supplementation (T2, T3, and T4) exhibited enhanced growth performance compared to the control group (T1). The highest body weight gain was observed for T4, with piglets gaining an average of 9.27 kg, significantly higher than the 8.15 kg gain recorded forT1 ($P < 0.001$). Piglets ofT3 and T2 showed intermediate gains of 8.95 kg and 8.48 kg, respectively. Feed intake was at the same level across all groups ($P = 0.275$). The feed-to-gain ratio was notably improved in the phytase-supplemented groups. Piglets of T4 had a feed-to-gain ratio of 1.59, compared to 1.73 of T1 ($P < 0.001$). Piglets ofT3 (1.63) and T2 (1.65) groups also demonstrated a significant ($p < 0.05$) and tendential better feed efficiency than T1, respectively. The apparent praecaecal digestibility for Ca (39.0 % (T1), 43.0 % (T2), 46.9 % (T3), 50.9 % (T4) ($p<0.001$) and for P (38.0 % (T1), 43.4 % (T2), 48.1 % (T3), 51.6 % (T4)) ($p<0.001$) improved with increasing phytase levels. A similar pattern was found for total tract digestibility of Ca with 36.7 % (T1), 41,9 % (T2), 45.8 % (T3), 50.9 % (T4) ($p<0.001$) and P with 38.4 % (T1), 44.7 % (T2), 49.2 % (T3), 52.5 % (T4) ($p<0.001$), respectively. Praecaecal digestibility of crude protein tended to be improved with increasing phytase supplementation (69.3 % (T1), 72.0 % (T2), 72.7 % (T3), 73.3 % (T4) ($p = 0.091$)) whereas fat digestibility was not affected ($p=0.285$). Although the differences in apparent praecaecal amino acid digestibility were not statistically significant, a slight overall trend indicated improved amino acid utilization with increasing levels of phytase.

Conclusions: The results demonstrate that particularly at higher dose rates the bacterial hybrid 6-phytase increases digestibility of P and even enhances growth performance in weaned piglets, offering economic and environmental benefits by reducing the need for inorganic P sources but also for some other nutrients in the diet.

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63.

Performance, nutrient digestibility and gut function in weaned piglets fed mannanase supplemented feed

Leistungsparameter, Nährstoffverdaulichkeit und Darmfunktion bei Einsatz einer Mannanase im Futter von Absatzferkeln

Schulze Holthausen J., Männer K., Ebersbach L., Fickler A., Ader P., *Zentek J. – Berlin / Ludwigshafen

The role of mannans in animal nutrition has gained increasing attention due to their presence in plant-based feed protein sources, such as soybean meal (SBM), rapeseed meal (RSM), and sunflower meal (SFM). Mannans, a type of non-starch polysaccharide, are generally considered as indigestible by monogastric animals like pigs, due to the lack of endogenous enzymes breaking down these complex carbohydrates. As a result, mannans can interfere with nutrient digestibility and gut health, leading to reduced feed efficiency. Enzymes like endo-1,4-β-D-mannanase are designed to degrade mannans. These enzymes reduce the anti-nutritional effects, thereby enhance digestibility, and potentially support overall animal performance.

Methods: This study aimed to evaluate the effects of an endo-1,4-β-D-mannanase (Natupulse® TS, BASF SE, Germany) on growth performance, nutrient digestibility, and intestinal physiology of post-weaning piglets. A total of 32 weaned piglets were housed in pens with two animals and assigned to two dietary treatments over a 21-day trial period, following a four-day adaptation phase. The treatments consisted of a control diet based on corn, SBM, RSM and SFM and reduced nutrient density. The test diet was supplemented with the endo-1,4-β-D-mannanase (1080 TMU/kg). Key performance indicators such as body weight gain, feed intake, and feed-to-gain ratio were measured at weekly intervals. Additionally, apparent praecaecal digestibility of crude protein, amino acids, crude fat, calcium (Ca) and phosphorus (P) were assessed through ileal digesta sampling on days 21-24. Jejunal tissue samples were collected for electrophysiological measurements to assess physiological tissue function, specifically glucose-stimulated electrogenic transport, in Ussing chambers. The statistical evaluation was conducted using univariate ANOVA with significant differences declared at $P \leq 0.05$.

Results: Piglets in the mannanase group gained 8.72 ± 0.39 kg, compared to 8.15 ± 0.71 kg in the control group ($P = 0.069$). The feed-to-gain ratio was significantly improved by supplementation of mannanase (1.619 compared to 1.731, $P = 0.003$). The inclusion of the enzyme led to significant improvements in digestibility for both, crude protein and crude fat. The apparent praecaecal digestibility of crude protein increased from 69.2 % in the control to 73.8 % in the supplemented group ($P = 0.003$), while it increased for crude fat from 79.9 % to 83.2 % ($P = 0.041$). Apparent praecaecal Ca digestibility was rising from 39.0 % in the control to 41.8 % in the mannanase supplemented group ($P = 0.045$), and P digestibility increased from 38.0 % to 42.2 % ($P = 0.026$). Amino acids such as valine ($P < 0.001$), threonine ($P = 0.028$), and methionine ($P = 0.031$) showed significant increases in apparent praecaecal digestibility compared to the control group. Piglets of the mannanase group demonstrated an improvement in glucose-stimulated electrogenic transport ($P = 0.044$).

Conclusions: The supplementation of endo-1,4-β-D-mannanase in piglet diets significantly improved feed efficiency and praecaecal nutrient digestibility. The results suggest that endo-1,4-β-D-mannanase could be a valuable additive in piglet feed consisting of mannan-containing feed ingredients.

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64.

Adaptation of rumen fermentation processes, the ruminal microbiome, and digestibility in response to the supplementation of willow leaves (*Salix spp.*) in cattle nutrition

Anpassung der Pansenfermentationsprozesse, des Pansenmikrobioms und der Verdaulichkeit als Reaktion auf die Supplementierung von Weidenblättern (Salix spp.) in der Rinderfütterung

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Tannin-rich willow leaves have been studied as feed supplement for cattle grazing on pastures. Previous studies have shown that tannins from various trees may inhibit rumen fermentation processes and alter the rumen microbiome [1]. However, the effects of tannin-rich willow leaves (*Salix spp.*) on the rumen fermentation, microbial adaptation, and digestibility in cattle have not been investigated. Therefore, the objective of this study was to elucidate the rumen environment, the rumen microbial composition, and total tract digestibility in cattle supplemented with willow leaves.

Methods: Eight weaned German Holstein bull calves kept on pasture were supplemented with concentrates and willow leaves (SAL) or alfalfa hay (CON) in a crossover design. Both supplements were formulated isoenergetic (9.7 MJ ME/kg DM) and isonitrogenous (134.5 g crude protein/kg DM), providing 45.8% of DM intake, assumed that the total DM intake amounts to 2.4% of the body weight. After 2 weeks of adaptation to SAL or CON, calves were housed in tie stalls. Rumen fluid was sampled via oral tubing. Blood samples were taken from the V. jugularis and analyzed for allantoin and uric acid concentrations by HPLC. The intake of grass clippings and SAL or CON and fecal excretions were recorded and sampled for 4 days. Fecal N (Kjeldahl), fecal aNDFom concentrations (amylase treated), and feed nutrients were determined in pooled samples by LUFA GmbH (Rostock, Germany). Rumen fluid was analyzed for NH3 using the Conway method, for H_2 [2], and for short-chain fatty acids (SCFA) by gas chromatography. Individual SCFA concentrations were calculated as mol% of the total. The rumen microbial composition was determined in ruminal purified DNA using 16S rRNA sequencing. The results of the latter were then taxonomically assigned using mothur (1.44.1) and the Silva database (rel. 138), and analyzed in R. The remaining results were analyzed in a MIXED MODEL in SAS (9.4; SAS Institute Inc., USA) including the repeated statement diet and the fixed effects diet (SAL or CON) and block of sampling (n = 4).

Results: Dry matter intake (4.91 ± 0.28 kg/d) and average daily gain (1.24 ± 0.06 kg/d) were comparable between groups. The sum of plasma allantoin and uric acid concentrations, a marker of microbial protein synthesis, was 6% higher in SAL-fed than in CON-fed calves ($P < 0.05$). The N digestibility was 8.7% lower in SAL-fed calves, whereas aNDFom digestibility was comparable between groups. The NH3 concentration in rumen fluid was 54% reduced in SAL-fed compared to CON-fed calves ($P < 0.01$), whereas the ruminal H_2 concentration was comparable between groups. The SCFA analysis revealed a lower acetate/propionate ratio (5%; $P < 0.05$), iso-butyrate proportion (0.001 mol%; $P < 0.1$), and valerate proportion (0.007 mol%; $P < 0.001$) in rumen fluid of SAL-fed calves compared to CON-fed calves. Taxonomic analysis revealed that eight fiber-degrading rumen microbial taxa were more abundant in CON-fed than in SAL-fed calves, accounting for 10 and 6% of the rumen microbial community, respectively. Three fiber-degrading taxa were more abundant in SAL-fed than in CON-fed calves, accounting for 4.3 and 2.8%, respectively. We found three taxa associated with denitrification function and three taxa associated with nitrogen fixation more abundant in SAL-fed than in CON-fed calves. The genus Butyrivibrio 2 was 56% more abundant in SAL-fed than in CON-fed calves ($P < 0.1$) and correlated negatively with the N digestibility ($r = -0.52$; $P < 0.05$) and positively with aNDFom digestibility ($r = 0.51$; $P < 0.05$).

Conclusions: Our results suggest that willow leaf supplementation to cattle affects rumen fermentation processes and the proportion of rumen microbial taxa involved in proteolytic, denitrifying, nitrogen-fixing, and fiber-degrading processes. These ruminal adaptations may be involved in maintaining the level of microbial protein synthesis despite a reduction in total tract N degradability in willow leaf supplemented cattle.

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65.

Effects of different concentrations of grape marc extract on growth performance and apparent praecaecal nutrient digestibility of 35-day-old broiler chickens

Auswirkungen verschiedener Konzentrationen von Traubentresterextrakt auf die Wachstumsleistung und die scheinbare praecaecale Nährstoffverdaulichkeit von 35 Tage alten Masthühnern

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The ban on antibiotic feed additives has spurred the search for plant-based alternatives. White grape marc, a by-product of winemaking processing, is rich in bioactive compounds, mainly polyphenols, with potential antimicrobial activities as well as antioxidant and anti-inflammatory properties [1, 2]. The optimal dosage of a grape marc extract as a poultry feed additive, has not been determined. This study aimed to evaluate the effects of different doses of a white grape marc extract on performance and apparent praecaecal digestibility in broiler chickens to determine efficacy and the optimal effective dose.

Methods: 400 healthy male broiler chickens (Cobb 500) were evenly distributed into 40 pens according to their body weight (BW). The experimental design included 4 treatment groups, each containing 10 replicates. The control group (CON) received no white grape marc extract, while the treatment groups LPP, MPP, and HPP were supplemented with increasing extract concentrations that provided low (200 mg/kg), medium (750 mg/kg), and high concentration (1500 mg/kg) of polyphenols, respectively. From day 0 to 11 broilers received a starter diet in mash form and from day 12 to 35 a pelleted grower diet. The grower diet was supplemented with Celite as inert marker for determining the apparent praecaecal digestibility of nutrients. The average pen BW and feed intake (FI) were recorded on days 11, 21 and 35 to calculate average daily FI, BW gain and feed conversion rate. On day 35, ileal digesta was collected (a pool of three birds/pen) to determine the apparent praecaecal digestibility of different nutrients. Statistical analysis was performed using one-way ANOVA followed by post-hoc Tukey test. With non-normally distributed data, the Kruskal-Wallis-Test followed by Mann-Whitney-U-Test was used (significance at $p<0.05$).

Results: The different concentrations of white grape marc extract had no effect on the growth performance of the broiler chickens. Additionally, the apparent praecaecal digestibility of crude protein, crude ash, calcium, phosphorus, and most amino acids remained unaffected. However, the digestibility of aspartic acid increased with higher grape marc extract levels. As a result, the MPP and HPP groups exhibited a higher apparent ileal digestibility (AID) of aspartic acid (80.6% and 80.9%, respectively) compared to the LPP group (76.3%) ($p=0.006$), while the differences with the control group were not significant.

Conclusions: The different concentrations of white grape marc extract used as feed additive in broiler diets did not have an impact on growth performance or praecaecal digestibility of several nutrients of 35-day-old broilers and, importantly, did not produce adverse effects. While previous studies indicate that grape marc extract may positively influence growth performance [3], further research under suboptimal conditions would be useful.

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66.

Impact of mixture of essential oils and vitamins on growth and immunity performance in shrimp *(Litopenaeus vannamei)*

Auswirkungen einer Mischung aus ätherischen Ölen und Vitaminen auf das Wachstum und die Immunität von Shrimp (Litopenaeus vannamei)

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The whiteleg shrimp *(Litopenaeus vannamei)* is currently regarded as one of the most prevalent and valuable cultured aquatic species. However, its production in intensive aquaculture systems has made it vulnerable to Vibrio parahaemolyticus (VP), which causes acute hepatopancreatic necrotic disease (AHPND). With the restriction on antibiotic use, some alternatives, like essential oils, are being utilized to protect these animals from bacterial infections. The objective of this study was to assess the impact of two supplementary products on enhancing growth performance and shrimp immunity against VP.

Methods: In this study, Miarom Classic L®, which includes a blend of essential oils (e.g., eucalyptus, peppermint and anise) at dosages of 1.0 g/kg (M1) and 3.0 g/kg (M3), as well as Livervital L®, containing a mixture of liver supporting compounds (e.g., L-carnitine, choline chloride and betaine) at levels of 2.0 g/kg (L2) and 4.0 g/kg (L4), were sprayed separately or in combination (2.0 g/kg Miarom Classic L® & 3.0 g/kg Livervital L®, referred to as M2L3) on a commercial shrimp feed which was used as the control. All feeds were coated with 2% fish oil. The study was conducted in two separate phases. In the first phase, which lasted 8 weeks, a total of 90 shrimp with an average initial weight of 4.1 ± 0.08 g (Mean ± SD) were randomly assigned to three tanks as replicates for each treatment. The shrimp were fed five times daily. Feed residues and mortalities were recorded daily, while the group body weight per tank was measured in the beginning, every two weeks and at the end of study phase. During the research phases, the water temperature and salinity were 28.6 °C and 20 ppt, respectively. The growth parameters were measured in the first phase. A bacterial challenge study was conducted in the second phase on the shrimp from the first phase (average weight: 35.6 ± 1.03 g). In this phase, there were two control groups: negative and positive. The shrimp in both groups were fed commercial feed. However, unlike the negative control group, the shrimp in the positive one, along with the test groups that received feed additives, were exposed to a bacterial challenge. The immune response was evaluated in two ways. In the first one, four shrimp per tank were injected with VP_{AHPND} at an LD50 value of 10^4 CFU/ml to observe bacterial clearance in the hepatopancreas (HP) at 12 hours post-injection. In the second challenge method, 15 shrimp for each tank were infected by inoculating the rearing water with VP_{AHPND} at an LD50 value of $10^{6.5}$ CFU/ml and reared for 7 days. The mortality rate as well as phenoloxidase (PO) and respiratory burst (RB) activities in the haemolymph were measured in three shrimp out of those 15 animals at 48 hours after this challenge. The mean values for each treatment were statistically analyzed, and a significance level of $p<0.05$ was adjusted.

Results: No statistically significant differences ($p>0.05$) in growth parameters across the feeding groups were observed. However, the variation in weight gain and feed conversion ratio for the M2L3 and M3 groups were notably less than in the other groups during the first phase. In the subsequent phase, the bacterial density in HP showed a decreasing trend with increasing the inclusion of Miarom Classic L®, which was not statistically different to the negative control after intramuscular injection of VPAHPND. The PO in the M3 (0.1 ± 0.04) and L4 (0.1 ± 0.01) groups was statistically identical to that of the unchallenged control (0.1 ± 0.03). RB activity in the L2 (1.4 ± 0.17) and M3 (1.3 ± 0.14) feeding groups was also statistically not different to the negative control (1.4 ± 0.15). In the second phase, the survival rate for all the test groups was statistically identical to the unchallenged shrimp, except for L2.

Conclusions: The inclusion of Miarom Classic L®, whether used alone or in combination with Livervital L®, in shrimp feed can boost growth performance and immunity as an eco-friendly approach.

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67.

Evaluation on the immunomodulatory potential of the probiotic - OmniBiotic Cats&Dogs - on equine peripheral blood mononuclear cells *in vitro*

Untersuchung des immunmodulatorisches Potenzial des Probiotikums „OmniBiotic Cats&Dogs“ auf equine periphere Blutmononukleäre Zellen in vitro

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Probiotics are widely recognized for their beneficial effects on gut health and immune function. OmniBiotic Cat & Dog is a licensed probiotic product, developed primarily for cats and dogs, containing two strains: *Enterococcus faecium* DSM 10663 / NCIMB 10415 and *Lactobacillus acidophilus* CECT 4529. Although *E. faecium* has been studied in foals, where its supplementation did not reduce the incidence of diarrhea and, in some cases, prolonged it [1], its potential to modulate immune responses, especially in adult horses, remains an area of interest. In pigs, *E. faecium* has been shown to influence adaptive immune responses, particularly by enhancing cytotoxic T-cell activity [2]. These findings highlight that while its effects on gastrointestinal health may vary, *E. faecium* could still play an important role in immune regulation. Consequently, this study aims to investigate whether the probiotic formulation in OmniBiotic Cats & Dogs can modulate immune responses in horses, focusing in a first approach on its effects on equine peripheral blood mononuclear cells (PBMCs) as a model. Subsequently, we hypothesize that the probiotic formulation exerts direct immunomodulatory effects on adaptive immune cells from blood drawn from adult horses.

Methods: Blood samples were collected from seven horses into EDTA-Vacutainers and immediately stored on ice. PBMCs were isolated from the blood samples using density gradient centrifugation with Histopaque in a 2:1 ratio. Three trials were conducted with two to three horses per trial, following the same procedure each time. 5 x 10^5 PBMCs were cultured in RPMI or DMEM media with different serum substitutes (10 % of fetal bovine serum, horse serum, or FC1) in a 24-well plate, and their growth, proliferation, and survival were monitored using the Incucyte S3 and Countess III systems. PBMCs were treated with 2,5x10^5 cfu of the product OmniBiotic Cat & Dog. A negative control without treatment and a positive control with a Concanavalin A (10 µM) treatment to control for the ability to response of the primary isolated immune cells was included. Immune cell subsets, including CD4, CD8, Mono-APC, and IL-17, were identified through antibody staining and visualized using immunofluorescence and flow cytometry with the BD Canto. Statistical analyses were performed using the Wilcoxon rank-sum test to evaluate differences between groups.

Results: PBMCs cultured in RPMI medium demonstrated superior survival rates compared to those cultured in DMEM ($p < 0.05$). Fetal bovine serum (FBS) was the most suitable serum substitute, outperforming both horse serum and FC1 serum ($p < 0.05$). Despite some individual differences, the probiotic exhibited potential positive immunomodulatory effects on PBMCs under optimal growth conditions ($p < 0.05$, in RPMI medium with FBS). Successful visualization of immune cell subsets was achieved, though further refinement is needed for certain fluorescent markers.

Conclusions: The findings of this *in vitro* model suggest that „OmniBiotic Cats&Dogs“ has the potential to directly modulate equine PBMCs when cultured in suitable conditions, particularly with RPMI medium and FBS. Further research, including *in vitro*, as well as *in vivo* studies, is necessary to explore the full potential of this probiotic formulation as an immunomodulatory agent in horses. To reduce experiments involving animals, we are developing refined assays to assess the immunomodulatory potential of these probiotics more closely to the *in vivo* situation using equine colon organoids to mimic the intestinal barrier. We aim to present the first results of these additional assays at the GfE Tagung 2025.

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68.

Effect of the health and parity of sows on the composition of the colostrum

Einfluss von Gesundheit und Parität von Sauen auf die Zusammensetzung des Kolostrums

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Colostrum is the primary source of nutrients and proteins for newborn piglets. To give each piglet the best possible start in life, it is important to assess the quality of the colostrum. This can be achieved by analysing its ingredients, as these are key indicators of quality. The aim of this study was to investigate how the composition of colostrum relates to the health and parity of the sow, especially with regard to dry matter, crude protein, and fatty acid pattern.

Methods: Data were collected in the EIP-project Select4Milk with the registration number 276034540350521. The presented study was conducted on a farrow-to-feeder farm. The farm housed around 500 hybrid sows (db.Viktoria, BHZP Large White x BHZP Landrace). There was no change in the sows‘ feed during the experimental period. 187 of the sampled sows were clinically examined shortly after farrowing (within 2 days at most), as detailed described by Rosengart et al. (2021) [1]. Shortly, if the sow was reluctant to stand up, if the sow had purulent discharge, if the sow did not eat or if the piglets were given a rating of 1 (few injuries on carpal joints), each finding was awarded one point. Two points were allocated if the rectal temperature was ≥39 °C and ≤39.4 °C or if the piglets were given a rating of 2 (many injuries on carpal joints). If the rectal temperature was ≥39.5 °C and ≤39.8 °C, four points were given. Five points were awarded if the temperature was ≥39.9 °C. If a sow was given a total of zero to two points, she was considered healthy (n = 83), in the case of three to five points, clinically suspicious (n = 74), and when allotted more than five points, diseased (n = 30). Clinical data were only collected from sows of 26 farrowing groups, the parity from sows of 41 farrowing groups. To evaluate the data according to parity, the sows were divided into four parity groups: Group 1 with n = 93 (parity 1 and 2), Group 2 with n = 109 (parity 3 and 4), Group 3 with n = 61 (parity 5 and 6), and Group 4 with n = 71 (parity 7 or higher). Within first 6 hours after farrowing a single colostrum sample was collected (5 to 10 samples per group). Instantly after collection, the samples were frozen at -20 °C. The colostrum samples were analysed for dry matter (according to VDLUFA), crude protein content (according to VDLUFA), and fatty acid pattern [2]. From fatty acid pattern, the total fatty acids were calculated. The statistical analysis was carried out using SAS Enterprise Guide® (ANOVA).

Results: The study found that the dry matter content, the content of crude protein, the total fatty acids, and the n-6:n-3-ratio in the colostrum did not differ significantly between sows with different health status shortly after farrowing ($p>0.05$). The colostrum showed no significant differences in total solid content across the parity groups (in g/kg original substance: 224 ± 30.99, 224 ± 38.61, 217 ± 34.56, 214 ± 33.65 ($p>0.05$)). Similarly, the crude protein content did not vary significantly between the groups (in g/kg original substance: 146 ± 25.65, 144 ± 36.63, 146 ± 30.50, 144 ± 32.95 ($p>0.05$)). In contrast, the colostrum of sows in parity groups 1 (parity 1 and 2) and 2 (parity 3 and 4) had higher levels of total fatty acids compared to those in parity groups 3 (parity 5 and 6) and 4 (parity 7 or higher) (in g/kg original substance: 38.19a ± 17.47, 38.11^{a} ± 15.80, 30.59^{b} ± 12.75, 30.19b ± 13.55 ($p<0.05$)). The n-6:n-3-ratio also showed significant differences between the parity groups (14.6^{b} ± 1.8, 15.1^{ab} ± 1.6, 15.4^{a} ± 1.7, 15.5^{a} ± 1.5 ($p<0.05$)).

Conclusions: The findings suggest that the health of the sow shortly after farrowing has little to no influence on the composition of the colostrum. This is because it can be assumed that an infection of the mammary gland usually occurs after colostrum production. Furthermore, parity significantly affects the fat composition of the colostrum. As parity increases, the proportion of n-3 fatty acids tends to decrease, while n-6 fatty acids become more dominant.

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69.

Effects of varying fat concentrations and sources in a diet on the fecal microbiota of healthy adult cats

Einfluss variierender Fettgehalte und -quellen im Futter auf die fäkale Mikrobiota von gesunden adulten Katzen

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Based on research in human medicine, the dietary fat intake might affect the composition and metabolic activity of the gut microbiota by different mechanisms. These mechanisms are not completely understood so far, but antibacterial and bacteria-promoting effects are discussed, as well as an impact on the microbiota secondary to a modulation of the immune function [1,2]. Those effects are not only dependent on the amount of dietary fat, but also on the type of fatty acids, i.e., their saturation, chain length and double bond position [2]. In the present study, the impact of varying fat intakes on the intestinal microbiota were evaluated in healthy adult cats.

Methods: Ten healthy adult European Shorthair cats (7 female neutered, 3 male neutered, 46.6 ± 14.1 months old, initial body weight 4.99 ± 0.91 kg) received a commercial complete low fat diet (9 % crude fat in dry matter) without (control treatment) or with the addition of sunflower oil, fish oil or lard. The oils and lard were supplemented at a concentration of 0.5 g and 1 g per kg body weight and day, resulting in an average dietary crude fat concentration of 12 % and 15 % in dry matter, respectively. Each cat received each dietary treatment, using a randomized cross-over design. A supplementation period was divided into a 16-day adaptation period and a following 5-day collection period. The cats were single housed during the collection periods to allow for an individual collection of fecal samples. The study was approved by the relevant local authority (Regierungspräsidium Gießen, ethical approval code G41/2022). The fecal microbiota was analyzed using 16S rDNA sequencing and microbial metabolites using standard laboratory methods (lactate and biogenic amines: high-performance liquid chromatography; short-chain fatty acids: gas chromatography; ammonium: colorimetrically). For the statistical data analysis, the sunflower oil, fish oil and lard treatment were separately evaluated with SPSS 28 (descriptive analysis) and SAS 9.4 (repeated measures ANOVA and pairwise comparisons with Bonferroni correction; 3 doses: control treatment, 0.5 g and 1 g oil/fat per kg body weight and day). The level of significance was set at $P = 0.05$.

Results: No influence of the dietary fat level or source could be detected on the alpha-diversity metrics (Richness, Shannon Index, Evenness) and the relative abundance of dominant bacterial phyla and genera in the feces of the cats. In addition, the fecal concentrations of total short-chain fatty acids (means: 148-218 µmol/g), D-lactate (0.11-1.00 µmol/g), L-lactate (0.01-0.86 µmol/g), ammonium (35.6-45.4 µmol/g) and total biogenic amines (22.1-29.7 µmol/g) did not differ among the groups.

Conclusions: The gut microbiota of healthy adult cats was not affected by low to moderate fat concentrations or the n-6:n-3 ratio in the diet. Therefore, no adverse effects on a balanced microbiota can be assumed by moderately varying fat intakes in healthy felines. On the other hand, potential beneficial effects, particularly by the supplementation of immunomodulatory n-3-fatty acids, were also not detected on the healthy gut microbiome of cats.

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70.

Effect of canning as a common processing step on the solubility of phosphate in petfood

Einfluss des Kochprozesses in der Konservenherstellung auf die Löslichkeit von Phosphat in Futter

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Restricted use of phosphate (P) containing additives, i.e. inorganic P (Pi), in food and feed is recommended because of their potential adverse health effects [1]. However, the labelling of P additives is not mandatory, even though their use in commercial pet food is very common. The aim of this study was to evaluate possible effects of a processing method commonly applied in industrial wet pet food production on the amount of highly soluble P in different preparations, as measured by the method of Lineva [2]. This method differentiates the P fractions soluble in water or slightly acidic solution after 1 and 90 minutes.

Methods: As a first step, a diet containing lamb heart, liver, and kidney as well as wheat flour, water, linseed oil, sunflower oil, and cellulose was prepared by grinding and mixing all ingredients homogenously. The basic diet without any additions was sampled as well as the basic diet with a balanced premix without Pi. Sixteen varieties with different concentrations and combinations of Pi were produced by mixing the basic diet with an adapted premix (Pi sources: H_3PO_4, $NaH_2PO_4*H_2O$, KH_2PO_4, $CaHPO_4$; Ca/P-ratio: <1; 1-2; >2), to resemble the broad variety of pet food on the market. A subset of each variety was analysed without further processing (raw) while the rest was canned and cooked for 280 minutes at 121°C and 1 bar pressure. All samples were analysed in duplicates for solubility of P [2], total P, and calcium using standard methods (flame emission spectrometry and photometry). For comparison of raw and cooked samples, the P fraction soluble after 1 minute in water was considered (Psol1). Differences between raw and processed samples were analysed via paired t-test after testing for normal distribution (Shapiro-Wilk).

Results: Because of the high solubility of Pi, the amount of Psol1 is expected to be roughly the same as the added Pi. This correlation could be proven in the raw samples (R^2=0.99). However, this was not true for the cooked samples, where the correlation was far less pronounced (R^2=0.37). Therefore, the difference between the raw and pressure-cooked samples was significantly different ($p<0.001$). This is in line with the findings of Lineva et al., where cooking of animal and plant-derived material decreased the fraction of Psol1 [2]. This effect on the fraction of Psol1 was not only confirmed in this study, but an even stronger effect on Psol1 was found through the combination of cooking and adding Pi. There is no distinct effect of different Pi sources on P solubility after pressure cooking, but an effect of a higher P concentration was very visible in the results. A potential explanation for the decrease in P solubility after pressure-cooking is that some Pi sources themselves can denature, aggregate, and change the secondary structure of proteins [3]. Chelation with other minerals from organic material or the premix is also possible. There was no correlation between the different Ca/P ratios and the measured amount of Psol1.

Conclusions: Laboratory scale testing of the amount of Psol1 before and after canning a moist home-prepared diet with and without added Pi sources revealed a reduction of the P solubility after 1 minute in water through pressure cooking. The method of Lineva et al. is used to measure the amount of highly soluble P in pet food, which partly derives from Pi [2]. However, this study shows that the amount of added Pi in canned pet food is probably even higher than it can be expected by measuring Psol1.

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71.

The effects of different thermal-treated fishmeal on the performance of rainbow trout (*Oncorhynchus mykiss*)

Die Effekte von unterschiedlich thermisch behandeltem Fischmehl auf die Leistung von Regenbogenforellen (Oncorhynchus mykiss)

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Thermal treatment is an essential step during fishmeal (FM) processing [1], with most commercial FM being produced under thermal treatments between 110 and 120 °C. New processing methods suggest that a gentler processing (70 °C) would improve fish performance. Authors have reported that high thermal treatments (140 °C) reduce the digestibility of FM and of its amino acids in comparison to FM produced at 70 °C [2]. Nonetheless, the effects caused by different thermal treatments on fish performance are still unclear. We hypothesised that the higher thermal treatment the worse the performance of fish would be. The aim of this study was to evaluate the performance of rainbow trout (*Oncorhynchus mykiss*) fed different thermal-treated FM.

Methods: For this performance trial 300 rainbow trout (*Oncorhynchus mykiss*, 163 ± 0.75 g, mean and SEM respectively) were randomly assigned to 30 tanks (13 fish per tank, 150 L). The FM used was produced using commercial frozen Atlantic mackerel (Scomber scombrus), which was freeze-dried to produce a control FM. After lyophilization, part of the FM was heated at 70, 105, 140, or 175 °C for 1h, resulting in five thermal-treated fishmeal (Control, 70, 105, 140, and 175 °C). Each FM was used in a complete diet formulated according to the recommendations of the Nutrient Requirements of Fish and Shrimp [3]. Fish were fed once daily until satiation with the test diets for 60 consecutive days and group weighted every two weeks to evaluate performance. The statistical design was completely randomized with five treatments (FM) with six replicates each (water tanks). Data were analysed using the software R Studio (2022.12.0+353, R Development Core Team). An analysis of variance (ANOVA) and a parametric post hoc test (Tukey‘s HSD test) were carried out. Values for each feeding group were presented as least square means (LS means). Differences were assumed as significant at $P < 0.05$. Additionally, regression analyses were conducted to determine the optimal thermal treatment for FM based on fish performance parameters.

Results: Trout fed FM 70 °C had the highest body weight (BW) and body weight gain (BWG) (355 and 193 g, respectively), but no statistical differences were observed in comparison to trout fed FM Control (334 and 171 g), FM 105°C (333 and 171 g), and FM 175°C (350 and 183 g). Nonethelees, trout fed FM 70 and 175 °C had higher BW and BWG ($P<0.01$) than trout fed FM 140 °C (325 and 169 g, respectively). Moreover, trout fed FM 70 °C had higher ($P<0.05$) FI (224 g) than trout fed FM Control (188 g), FM 105°C (181 g), and FM 140 °C (196 g), which did not differ among each other ($P>0.05$). On the other hand, trout fed FM 175 °C had a statistically similar FI (216 g) to trout fed FM 70 °C. No differences were observed for FCR, but trout fed FM 105 °C showed a tendency ($P=0.69$) to have better FCR than trout fed FM 175 °C. Based on the regression analyses, it was observed that trout fed FM 70 °C presented an optimal BW and BWG. Additionally, trout performance worsened as thermal treatments increased up to 140 °C; nonetheless, these effects were not observed for trout fed FM 175 °C, which led the authors to assume a physiological adaptation of the fish to better absorb the FM nutrients.

Conclusions: The current study showed that rainbow trout (*Oncorhynchus mykiss*) performance is susceptible to FM produced under different thermal treatments. Trout fed FM 140 °C had the worst performance, meanwhile trout fed FM 70 °C presented the optimal BW and BWG among treatments. Nevertheless, trout fed FM 175 °C surprisingly presented a similar performance to trout fed FM 70 °C. The authors suggest that more trials should be conducted to better evaluated possible physiological adaptations of the trout to better absorb the nutrients of ingredients that have undergone high thermal treatments.

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72.

Does inactivation of Deoxynivalenol by hydrothermal treatment with sodium metabisulfite alter nutrient composition of maize?

Verändert die Inaktivierung von Deoxynivalenol durch hydrothermische Behandlung mit Natriummetabisulfit die Nährstoffzusammensetzung von Mais?

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Deoxynivalenol (DON) is one of the main mycotoxins produced by several Fusarium species occurring on grains and may negatively affect feed intake and growth rates especially in pigs when critical concentrations are exceeded. Hydrothermal treatment with sodium metabisulfite (SBS) of grains that are contaminated with DON is one method that has been shown to effectively inactivate DON [1]. Optimized conditions of such treatment include the addition of 3 % steam which resulted in an increase in temperature of 50°C and 0.03 g/kg moisture [2]. As such conditions may affect the composition or quality of nutrients it is important to gain knowledge on such effects in order to assure the applicability in practice. Therefore, the present study aimed to investigate the effects of the inactivation of DON by hydrothermal treatment with SBS on the nutrient composition of DON contaminated maize.

Methods: A batch of DON-contaminated maize kernels was divided and half of the batch was ground to maize meal. Those two sub batches were further divided into 15 portions, 5 served as starting material which was not treated, 5 portions were hydrothermally treated without the addition of SBS and 5 portions were treated with the addition of SBS. The treatment was carried out according to the previously optimised settings with 3 % steam and a treatment time of 10 seconds with 10 g SBS/kg in an experimental conditioner [2]. All samples were analysed for crude ash, crude protein, crude fat, sugar, starch and the degree of starch gelatinisation [3]. Analysis of variance in the 2x3 2-factorial design (matrix: kernels or meal; treatment: untreated, hydrothermally treated without and with SBS) was performed using Statistica (Version 14.0.0.15).

Results: The contents of crude protein, crude fat and sugar were not affected by any of the treatments. The ash content of the SBS treated samples was increased by 5.9 g/kg ($p_{treatment}<0.001$) as compared to the starting material, which equals the addition of 10 g SBS of which Na and S comprise 58 % of the molecular weight. The content of starch was reduced by 0.94 % in maize meal as compared to kernes ($p_{matrix}=0.001$) and it decreased further by 1.2 % by the hydrothermal treatment and 2.2 % by the hydrothermal treatment with SBS ($p_{treatment}<0.001$). Concerning the degree of starch gelatinisation, the tested matrices were differently affected by the treatment ($p_{matrix\ x\ treatment}<0.001$). While in maize meal the degree of starch gelatinisation increased by 32-34 %, no changes of starch gelatinization were observed in maize kernels.

Conclusions: The present results suggest that hydrothermal treatment with SBS to inactivate DON may alter the starch content and quality. However, as starch gelatinisation is beneficial for the digestibility by monogastric animals, these changes in quality may compensate the minor losses in quantity. Further investigations should evaluate the observed effects in further matrices (e.g. wheat) which are relevant for DON inactivation.

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73.

Adverse health effects of sodium and potassium phosphate addition to the diet of fattening pigs

Gesundheitsschädliche Auswirkungen des Zusatzes von Natrium- und Kaliumphosphat zum Futter von Mastschweinen

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It has been demonstrated in several species that oral intake of phosphate (P) salts from phosphate-containing food and feed additives can have adverse heath effects, e.g., on the kidneys, the cardio-vascular system, and the skeleton [1,2,3]. The study aimed to investigate certain effects of surplus amounts of highly soluble inorganic P in pigs, and therefore the possible suitability of the pig as a large animal model for phosphate toxicity.

Methods: Fourteen castrated boars (Danish landrace) aged 3 months were allocated (weight-matched) to control (CON; n=6) and high P diet (HP; n=8). Sodium and potassium phosphates (NaH_2PO4, KH_2PO_4) were added to diet HP, increasing the P concentration from 0.5g/MJ ME in diet CON to 2.2g/MJ ME. The Ca/P ratio amounted to 0.4/1. After 12 to 13 weeks, blood samples were taken for serum Ca and P measurements. Afterwards, the animals were sacrificed, and necropsies were performed. Kidneys, humeri, and metacarpi were sampled, i.a., for Ca and P analysis, histology, and micro-computed tomography (µCT). For statistical analysis, Student‘s t-test or Man-Whitney rank sum test were used after testing for normal distribution (Shapiro-Wilk).

Results: Circumference, absolute and relative weight of the kidneys per kg metabolic bodyweight was significantly increased in group HP ($p<0.001$ to 0.008). Kidneys of HP pigs were lighter in colour with a fine-grained surface. Histological examination showed no systematic alterations in the kidneys of CON pigs while in the kidneys of HP pigs multifocal, expansive mineralisation in numerous tubules and collecting ducts with compression and partly destruction of epithelial tubule structures and atrophy of nephrons, as well as multifocal and confluent interstitial fibrosis was verified. The kidneys of the animals with P excess showed a clear phenotype with a large number of calcification foci in the µCT, while the kidneys of the control animals were free of them. In the humerus biopsy, the proportion of bone volume to total volume was significantly reduced in the HP group (CON 89.4±3.1 vs HP 81.4±5.3 %, p=0.007). In the biopsy of the metacarpal bones only a tendency was measured (CON 80.9±10.2 vs HP 75.1±11.2 %, p=0.108).

Conclusions: The results demonstrate that even in growing pigs with above-maintenance requirements of P, excessive intake of soluble P salts, especially with a narrow Ca/P ratio, as often found in processed foods and beverages, for only 3 months caused significant detrimental effects on kidney and bone health. The suitability of this large animal model of phosphate toxicity from commonly used phosphate containing food and feed additives for human medicine should be further elucidated.

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74.

Liver hemorrhages and keel bone fractures in experimental cohorts of two high-performing laying hen strains from week 30 to week 42 of age

Leberblutungen und Brustbeinfrakturen in Versuchsgruppen zweier hochleistender Legehennenlinien von der 30. bis zur 42. Lebenswoche

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Slaughterhouse studies revealed, that liver hemorrhages and keel bone lesions commonly occur in high performing laying hens. Several risk factors associated with feeding, housing and production level have been identified [1,2]. The present study aimed to describe the occurrence and the severity of these lesions in experimental cohorts of laying hens under standardized rearing, housing and feeding conditions.

Methods: The study comprised 438 hens (Lohman Selected Leghorn (LSL) n = 220; Lohmann Brown Classic (LB) n = 218) that had been reared under identical management, feeding and housing conditions in a free run indoor barn. They were part of the interdisciplinary Research unit PFowl (https://p-fowl.uni-hohenheim.de/). Animals were slaughtered in four cohorts of 110 animals at 30, 34, 38 and 42 weeks of live. Four weeks before slaughter the respective cohort was moved to individual metabolic units, equipped with a wooden perch, a nest, a feeding trough, water cups, and a wire mesh floor. 100 animals (LB n = 50; LSL n = 50) were fed a diet without mineral P supplementation (P-) whilst ten animals, fed a diet with mineral P supplementation (LB n = 5; LSL n = 5) served as controls (P^+). After opening, the abdominal cavity was inspected for bleeding before the liver was removed. Livers were photographed and the bleeding score, considering size and number of lesions, was assessed. [1]. A subset (P-: LB n = 20, LSL n = 20; P^+: LB n = 5, LSL n = 5) was examined for keel bone fractures. Keel bones with adherent breast muscles were dissected from the carcass and examined by X ray. Fractures were scored using a visual analogous score [3]. This score is based on the amount of bone affected and the intensity of callus formation. After maceration, keel bones were ashed. Data were analyzed in a three-factorial analysis of variance including strain, diet and age.

Results: Out of 438 animals 9 % showed no bleeding lesion in the liver, 66% had a score of 1 (< 10 lesions > 0.8 cm) and 25 % (20% LB; 5 % LSL) had a bleeding score of 2 (> 10 lesions > 0.8 cm) or higher. Strain exerted a significant effect leading to higher scores in LB than in LSL hens ($P < 0.001$). Concerning keel bone fractures, 28% of the examined animals displayed a fracture score of 0, 36% a score of 1 and 36 % a score of 2 and higher up to 4 (4%). Fracture severity was significantly higher in week 42 (1.66 ± 0.15) than in week 30 (0.55 ± 0.15; $P < 0.001$). Withdrawal of mineral P led to higher scores in LB hens (1.70 ± 0.15 vs. 1.04 ± 0.23; $P = 0.024$) but not in LSL hens (1.03 ± 0.23 vs. 0.99 ± 0.15; $P = 0.993$). Cohort and diet significantly affected ash content. Ash content of keel bones was lowest (43.8 ± 0.47 % of DM) in week 34 and highest in week 38 (45.0 ± 0.47 % of DM). Controls consistently showed higher ash content (45.4 ± 0.23 % of DM) than animals without mineral P supply (43.1 ± 0.43 % of DM). Ash content was negatively correlated with fracture score ($\rho = - 0.27$; $P < 0.001$).

Conclusions: The present study also confirms the high prevalences of liver hemorrhages and keel bone fractures - currently reported in commercial laying hens - for the experimental cohorts studied herein. Strain exerted a significant effect on both scores, but lesions occurred in both strains. Thus differences rather concerned severity than general occurrence of these lesions. Regarding fracture scores it has to be considered, that callus formation and mineralization occur two to six weeks after the preceding insult, implying that fractures might have been already present, when P- diet was applied. Additionally, increasing scores over time might point to recurrent insults over time cumulating in week 42. Low correlation between ash content and keel bone fracture score further implies pathogenic mechanisms beyond bone mineralization that need further investigation.

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75.

Age- and strain-dependent fibroblast growth factor 23 (FGF23) and αKlotho expression in different organs from two laying hen strains

Alters- und linienabhängige Fibroblastenwachstumsfaktor 23-(FGF23) und αKlotho-Expression in verschiedenen Geweben zweier Legehennenlinien

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In humans and rodents, fibroblast growth factor 23 (FGF23) derived from bone is an important hormonal regulator of renal phosphate and vitamin D homeostasis and a disease biomarker. Its co-receptor αKlotho has emerged as a powerful anti-aging factor with versatile health benefits. Little is known about their role in poultry despite the great impact of phosphate homeostasis on bone integrity and eggshell quality. We aimed to analyze FGF23 and αKlotho gene expression in two common laying hen strains subjected to diets with standard and low phosphorus content.

Methods: The experiment consisted of a 2x2x2-factorial arrangement of treatments with the factors age, strain and diet (n=10 per treatment).15- or 20-week-old Lohmann Brown-Classic (LB) or LSL-Classic (LSL) hens were fed a standard maize-soybean meal-based diet containing 0 or 1 g/kg additional mineral phosphorus for 4 weeks. After sacrifice, gene expression was determined by qRT-PCR in different organs applying standard protocols. For statistical analysis, a 3-factorial analysis of variance using the MIXED procedure of SAS was applied.

Results: Independent of strain and diet, FGF23 bone expression was significantly lower (19-week-old hens: 0.150 ± 0.022 arbitrary units (a.u.); 24-week-old hens: 0.066 ± 0.008 a.u.; $p = 0.0001$) and hepatic FGF23 expression higher (19-week-old hens: 0.170 ± 0.015 a.u.; 24-week-old hens: 1.416 ± 0.062 a.u.; $p < 0.0001$) in 24-week-old than in the 19-week-old hens. In contrast, αKlotho expression in bone (19-week-old hens: 0.006 ± 0.001 a.u.; 24-week-old hens: 0.068 ± 0.009 a.u.; $p < 0.0001$), liver (19-week-old hens: 0.0020 ± 0.0004 a.u.; 24-week-old hens: 0.0112 ± 0.0029 a.u.; $p < 0.0001$), and kidney (19-week-old hens: 3.152 ± 0.217 a.u.; 24-week-old hens: 3.999 ± 0.257 a.u.; $p = 0.002$) was significantly higher in the older than younger animals. LSL hens exhibited higher hepatic αKlotho expression (LSL hens: 0.0118 ± 0.0027 a.u.; LB hens: 0.0011 ± 0.0002 a.u.; $p < 0.0001$) than LB hens irrespective of the diet and age. Surprisingly, dietary phosphate content did not significantly affect FGF23 and αKlotho expression.

Conclusions: Our study points to a strong impact of age and strain on FGF23 and αKlotho expression in two high performance laying hen strains, effects possibly associated with initiation of the egg production phase. Further studies are needed to elucidate the physiological relevance of the results.

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76.

Mineral content in digesta along the gastrointestinal tract of horses fed hay, hay with different levels of oats or kept on pasture

Mineralstoffgehalte in der Digesta entlang des equinen Verdauungstrakts bei Fütterung von Heu, Heu mit unterschiedlichen Haferanteilen oder Weidehaltung

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Mineral turnover in the digestive tract (DT) is affected by mineral origin and supply [1] as well as diverse conditions balancing absorption and secretion. For example, the expression of the Na-dependent SGLT1 transporter in the small intestine of horses is elevated by hydrolysable carbohydrates from oat grains [2]. The aim was to study mineral contents in the digesta along the DT using starch-containing vs forage only diets. Diet and anatomical section (anS) were hypothesised to lead to individual patterns of mineral content.

Methods: 23 horses were allocated to four diets: meadow hay ad lib. (HAY), meadow hay ad. lib. with one meal of oats/day at 1 or 2 g starch/kg BW (OS1 or OS2), or 24 h/day pasture (PST). Per kg dry matter (DM), oats contained 474 g starch, hay, PST and oats 86, 78 and 41 g water soluble carbohydrates respectively. Horses were provided with free access to water, salt lick and straw bedding within the stable. Intake of hay and salt lick was monitored, individual mineral intake was assessed. After > 34 days, 2 h postprandial, they were euthanised, dissected and digesta were sampled from 11 anS of the DT: stomach (pars nonglandularis, pars glandularis), jejunum (jej), caecum, colon ventrale dextrum, colon ventrale sinistrum, pelvic flexure, colon dorsale sinistrum (cds), colon dorsale dextrum, colon transversum and rectum (rec). Feed and digesta were analysed for dry matter (DM) and minerals (ICP OES). Statistical analysis was performed using SAS 9.4 (MIXED procedure) with period, anS and diet as fixed effects and animals as random effect.

Results: The feed contained the following mineral contents:pasture grass, in g/kg DM, Ca 5.8, P 2.6, Mg 1.7, Na 0.28, K 17.9; in mg/kg DM, Fe 276, Cu 5.5, Zn 35, Mn 56; hay, in g/kg DM, Ca 3.1, P 2.1, Mg 1.41, Na 0.57, K 20; in mg/kg DM, Fe 100, Cu 4.4, Zn 20, Mn 53; straw, in g/kg DM, Ca 2.2, P 0.17, Mg 0.62, Na 0.0, K 14; in mg/kg DM, Fe 47, Cu 3.4, Zn 3.8, Mn 20; oats, in g/kg DM, Ca 0.77, P 4.1, Mg 1.2, Na 0.12, K 7.0; in mg/kg DM, Fe 88, Cu 3.5, Zn 30, Mn 31. The average daily mineral intake through salt lick for all stabled horses amounted to 29 mg Na/kg$^{0.75}$ BW and 45 mg Cl/kg$^{0.75}$ BW. Overall mean DT mineral contents were as follows: in g/kg DM, Ca 2.5±1.1, P 4.3±1.2, Mg 1.4±0.46, Na 15.5±11.8, K 13.8±3.32; in mg/kg DM, Fe 304±108, Cu 5.7±1.7, Zn 43±16, Mn 86±16. They were affected by diet (except Na, Mn), anS and interaction of both (except Cu, Mn) ($P<0.05$), with mostly highest means in PST. Na was highest in jej and Mn in cds ($P<0.05$). Up to the jej, P was highest with oats (vs HAY $P<0.05$) and rose from caecum to cds (highest in PST) with subsequent overall decline until rec.

Conclusions: Results confirm the impact of diet and anS on digesta mineral contents. Na contents were widely independent from the diet, which is in line with [3]. Expected higher P with oat diets was obvious in the upper DT only. Ongoing analysis will show if diet-specific microbial enzyme expression impacts P release from phytate.

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77.

Effects of per- and polyfluoroalkylsubstances (PFAS) on the health of Suffolk sheep lambs

Einfluss von Per- und Polyfluoralkylsubstanzen (PFAS) auf die Gesundheit von Suffolk Schaflämmern

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous chemicals used in numerous everyday products, primarily as surfactants or surface protectants. These compounds comprise over 4,700 different substances, offering valuable properties for industrial applications but also represent a significant environmental concern. Research has consistently demonstrated that exposure to PFAS can have detrimental effects on human and animal health, including impacts on vaccine efficacy, lipid metabolism, liver function, and risk of cancer [1]. However, studies on the effects on farm animal health are scarce while especially grazing animals are easily exposed via feed, soil, and water. Therefore, this study aimed to investigate the effects of PFAS exposure on health parameters of Suffolk fattening sheep lambs.

Methods: The feeding trial was conducted at the Max Rubner-Institut and involved the rearing of 16 male fattening Suffolk lambs, which were orally supplemented with PFAS via a spiked sugar solution (perfluorobutanoic acid: 43 µg/kg body weight (BW), perfluoropentanoic acid: 51 µg/kg BW, perfluorobutane sulfonic acid and perfluorohexanoic acid: 49 µg/kg BW, perfluorohexanesulfonic acid: 10 µg/kg BW, perfluorooctanoic acid: 21 µg/kg BW, perfluorononanoic acid: 4 µg/kg BW, perfluorooctane sulfonic acid and perfluorodecanoic acid: 2 µg/kg BW) once daily by voluntary intake. At the start of the experiment the animals had an age of approximately 10 weeks and an average weight of 20 kg. They were assigned to one of four groups: Exp20 received PFAS for the entire 20-week period, Dep10 received PFAS for 10 weeks followed by a 10-week depuration period, Exp10 was exposed to PFAS for 10 weeks and euthanized thereafter and CON received the carrier material without PFAS. In week 12, the animals were vaccinated against bluetongue virus (BTV, Syvazul serotype 1, 4, and 8, Virbac Tierarzneimittel GmbH, Bad Oldesloe, Germany) and at the end of the trial animals were euthanized for necropsy. Blood samples were taken every second week from a jugular vein by needle puncture to analyze clinical chemistry (Indiko™ Plus, Thermo Fisher Scientific, Vantaa, Finland), hematology (ADVIA® 2120i, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA), and antibody titers against BTV (ID Screen® Bluetongue Competition ELISA, Innovative Diagnostics, Grabels, France). All data were statistically analyzed using the MIXED procedure for repeated measurements of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) with group, time, and their interaction as fixed factors. The values from the day before PFAS exposure were considered as co-variates. Each data set was analyzed twice, first including all 4 groups until week 10 and second including the three groups monitored over the 20 week period.

Results: The oral administration of the PFAS mixture had no significant effect on leukocytes, erythrocytes and derived indices of hematology over the whole trial. Only mean corpuscular volume (MCV), when it was calculated until the euthanasia of Exp10 (d 70), was differently affected by group over time ($p_{group*time} = 0.040$) while no significant effect was observed when the data were evaluated over the complete 20 weeks with the three groups ($p_{group*time} = 0.206$). Therefore, this variation may not be clearly assigned to PFAS exposure. The clinical chemical parameters, including electrolytes, indicators of protein and fat metabolism, and of liver and kidney health, do not provide clear evidence of a PFAS-induced effect. The vaccination against BTV resulted in induced antibody titers ($p_{time} < 0.001$) without influence of PFAS administration.

Conclusions: Under the conditions of the present study the PFAS exposure of the lambs did not result in clear effects on health as indicated by the evaluated parameters so far. Further investigations are required to substantiate this experimental outcome.

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78.

Investigation on phytate degradation during ensiling of freshly harvested soybeans with high-moisture maize

Untersuchung zum Abbau von Phytat während der Silierung von erntefrischen Sojabohnen mit Körnermais

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Phytate binds phosphorus in a form that is difficult to utilise for non-ruminant animals. The high levels of phytate in soybeans, therefore, reduce phosphorus availability and increase phosphorus excretion. Phytic acid also acts as a nutrient antagonist by binding other essential minerals. Ensiling high-moisture maize has been shown to increase phosphorus digestibility, as the degradation of phytate during fermentation releases phosphorus for absorption by the animal. Ensiling soybeans together with high-moisture maize may represent an innovative approach to reduce the need for drying and/or toasting the soybeans [1]. The objective of this study was to investigate the extent of phytate degradation during co-ensiling of soybeans and high-moisture maize.

Methods: Freshly harvested high-moisture maize (S0, 713 g DM/kg) and soybeans (S100, 869 g DM/kg), as well as their mixtures consisting of 15 % (S15), 30 % (S30), and 45 % (S45) soybeans, were ensiled in a laboratory silo test in a two-factorial experimental design in three replicates for 90 days in mason jars [1]. In addition to the untreated control, the effect of a biological ensiling agent with DLG action category 1 (improvement of the fermentation process: *L. plantarum*) and a biological ensiling agent with DLG action categories 1 & 2 (improvement of the fermentation process & increase in aerobic stability: *P. pentosaceus*, *L. brevis*, *L. buchneri*, *L. plantarum*, *L. rhamnosus*) were tested in all mixtures. The effect of a chemical silage additive (propionic acid: 5 l/t) was additionally examined in S15. The analysis of phytic acid (IP6) and inositol phosphate isomers (IP3-5) was performed chromatographically [2]. Results were analysed by ANOVA using GLM procedure of SAS 9.4 considering the effects of mixture (S0-S100) and treatment (control, silage additives).

Results: The IP6 concentration in maize and soybeans before ensiling was 11.3 and 20.1 µmol/g DM, respectively, with small amounts of IP5 near to the detection limit (0.3 µmol/g DM). A decreasing IP6 degradation with increasing soybean content was observed in the silages. Up to a soybean proportion of 30 %, IP6 was degraded entirely (detection limit: 0.1 µmol/g). In the S45 mixture silages, IP6 degradation was still 58 ± 1.1 %, whereas compared to the fresh material no significant changes could be observed in the pure soybean silages (S100). The concentrations of lower inositol phosphates in the silages were very low in S0 and S15, while in S30, IP3 and IP4 were detected and in S45, more IP5 was detected. Regarding phytate degradation, parallels can be drawn with the fermentation quality of the silages [1]. Preservation through lactic acid fermentation and corresponding pH reduction occurred only up to a soybean content of 30 %. Above this threshold, preservation was achieved through high dry matter content and the exclusion of oxygen. The application of silage additives, which were applied outside the optimal dry matter range, resulted in significant but only slightly decreased degradation of the lower inositol phosphates. Therefore, the degradation of phytic acid appears to have been primarily driven by the epiphytic microbiota, although plant intrinsic phytase activity may have also contributed to the process.

Conclusions: The degradation of phytic acid during the ensiling of high-moisture maize and soybean mixtures is significantly influenced by the maize content and, consequently, by the fermentation intensity of the silage. The percentage of IP6 degradation decreased as the soybean proportion in the silage increased. At soybean proportions of up to 30 %, degradation was highly effective. Moist ensiling of soybeans with maize may cause a higher phosphorus digestibility in pigs than dried soybeans, which should be the subject of further research.

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79.

Investigation of pyrrole-protein adducts following exposure to ragwort extract in a rat model

Untersuchung von Pyrrol-Protein-Addukten nach Exposition mit Jakobskreuzkrautextrakt im Rattenmodell

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Pyrrolizidine alkaloids (PAs) are naturally occurring toxins found in over 6,000 plant species [1]. In Germany, *Jacobaea vulgaris* (common ragwort), from the Asteraceae family is particularly significant for livestock, as animals may ingest PAs through contaminated forage such as silage or hay [1]. High concentrations of PAs can cause acute intoxication, while chronic exposure is linked to PA-induced hepatotoxicity. This study aimed to assess pyrrole-protein adducts (PPAs) as the biomarkers of PA exposure [2] and hepatotoxicity following administration of a ragwort extract in a rat model.

Methods: The experiment was conducted using male Sprague Dawley rats (n=35), aged 8 (+/- 1) weeks. The rats were divided into six equally sized groups, each receiving a single dose via oral gavage: a ragwort extract consists of 17 PAs and PANOs (Pyrrolizidine alkaloids N-oxides) (Erucifoline, ErucifolineNO, Jacobine, JacobinNO, Jacolin, JacolinNO, Jaconin, JaconinNO, Retrorsin, RetrorsinNO, Riddelliin, RiddelliinNO, Senecionin, SenecioninNO, Seneciphyllin, SeneciphyllinNO, Senkirkin), a purified PA extract, one of three individual PAs (jaconine, senecionine, seneciphylline), a purified PA extract, one of three individual PAs (jaconine, senecionine, seneciphylline), or a mixture of these three PAs. A control group received a slightly acidic solution of citric acid, the carrier substance for all other PAs. The dose administered to the extract group corresponded to 40 mg/kg body weight of total PAs, with other groups receiving proportional concentrations. After 24 hours, the animals were euthanized under isoflurane anesthesia by decapitation, and liver, plasma, and erythrocyte samples were collected for PPA quantification via UPLC-MS/MS. After oxidative cleavage with AgNO3 and subsequent derivatization with an Ehrlich-type reagent, PPAs were quantified using positive ESI-MRM with matrix-matched, internal calibration. Liver enzymes (ALT, AST, bilirubin, GGT) and histological evaluations were also performed.

Results: Liver samples showed the highest PPA concentrations in the group received the ragwort extract. Elevated PPA levels were also observed in the groups receiving the purified PA extract and the PA mixture. Among the individual PAs jaconine administration resulted in considerable PPA formation, while senecionine and seneciphylline yield lower levels. Preliminary plasma and erythrocyte PPA results indicate similar patterns across the groups. No differences were observed in liver enzyme levels between the groups, although initial histological evaluations revealed distinct patterns of liver degeneration.

Conclusions: Preliminary findings show that PPA levels in liver samples correlate with the extent of PA exposure and hepatoxicity. The highest PPA concentrations were observed following exposure to ragwort extract, suggesting greater hepatotoxicity compared to matrix-free PA exposure. These results underscore the importance of investigating PA exposure in livestock and further elucidating the toxicological impact of ragwort ingestion.

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80.

Responses of blood metabolite profiles to renunciation of mineral phosphorus in two contrasting high-yielding laying hen strains

Änderung des Metabolitenprofils im Blut durch diätetische Phosphorreduktion in zwei kontrastierenden hochleistenden Legehennenlinien

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Adequate phosphorus (P) supply is essential for laying hens and their diets are commonly supplemented with P from mineral sources. However, the P requirement appears to be lower than what is currently recommended [1]. A reduced mineral P supplementation in the diet increased endogenous phytase activity and phytate-P availability in broiler chicken [2]. Targeted metabolomics approach is a suitable tool for identifying diet-induced changes in metabolism. The aim of this work was to gain insight into the metabolic conditions of two laying hen strains without or with mineral phosphorus supplementation at different ages.

Methods: The experiment comprised a 2x2x2-factorial arrangement of treatments with the factors hen strain (Lohmann Brown-classic (LB), Lohmann LSL-classic (LSL)), hen production period (week 19, 24), and mineral P supplement (0 (P-), 1 (P+) g P/kg feed). Diets were based on corn and soybean meal and composed to contain all nutrients at recommended levels, except P. Exogenous phytase was not added. The analysed P concentrations were 3.3 and 4.4 g/kg. One offspring each of 10 nonrelated roosters per strain was placed individually in metabolic unit in a randomized complete block design (n = 10 hens per treatment) in week 15 and 20 of life and were fed one of the experimental diets. In weeks 19 and 24, all hens were slaughtered and blood plasma was collected. The concentrations of 188 metabolites in plasma were measured with Absolute-IDQ™ p180 kit by flow-injection tandem mass spectrometry and liquid chromatography mass spectrometry using a SCIEX 4000 QTRAP and a Xevo TQ-S Micro instrument with electrospray ionization, respectively. Metabolomics data were analysed by using MetaboAnalyst 6.0. The interesting metabolites were analysed in a 3-factorial analysis of variance using the MIXED procedure of SAS. The level of significance was set at $P < 0.05$.

Results: The average body weight and egg weight were higher in LB hens than in LSL hens (1613 g, 1299 g and 53 g, 49 g) but the average number of eggs in 29 days was not significantly different. The average body weight was lower in week 19 than in week 24 (1258 g and 1654 g). The factor diet exerted only a few effects overall. In week 24, carnitine (C0) and acetylcarnitine (C2) concentrations in plasma were higher ($P < 0.05$) in LSL hens fed P+ than in LSL hens fed P-. The C2 ($P < 0.001$), propionylcarnitine ($P < 0.05$) and trans-4-hydroxyproline ($P < 0.01$) concentrations were higher in hens fed P+ compared to hens fed P-. The factors age and strain did affect metabolite concentrations more than factor diet. Amino acids, amino acid derivatives and biogenic amines concentrations in plasma were decreased from week 19 to week 24. Hexose concentration was also decreased ($P < 0.001$). However, C2 ($P < 0.001$) and Ser ($P < 0.001$) concentrations were increased from week 19 to 24. In week 19, LB hens had a significantly higher C0 ($P < 0.001$) and kynurenine (Kyn) ($P < 0.001$) concentration in plasma and Kyn/Trp ratio ($P < 0.001$) than LSL hens. Nevertheless, Ser ($P < 0.001$) and Trp ($P < 0.001$) concentrations in plasma were significantly lower in LB hens than in LSL hens. In week 24, Kyn ($P < 0.01$) and symmetric dimethylarginine ($P < 0.001$) concentrations in plasma and Kyn/Trp ratio ($P < 0.001$) were significantly higher in LB hens than in LSL hens while, Cit ($P < 0.001$) and Ser ($P < 0.001$) concentrations were significantly lower.

Conclusions: Higher C0 and short-chain acylcarnitines in P+ groups suggested an influence of dietary P supply on metabolic pathways related to mitochondrial functionality and intracellular fatty acid transport. Age-dependent changes were associated with the onset of egg laying, reflecting the demand for egg protein synthesis. Strain-related differences implicate that LB and LSL hens adapted to the onset of egg laying differently.

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82.

Targeted amino acid profiling in the jejunal tissue of neonatal calves infected with *Cryptosporidium parvum* using LC-MS/MS

Zielgerichtete Aminosäuremessung mittels LC-MS/MS in der Dünndarmmukosa neugeborener Kälber, die mit Cryptosporidium parvum infiziert wurden

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Amino acids (AA) are essential players in the development of the immune system, inflammatory responses, integrity of the intestinal barrier, and proliferation of epithelial cells [1]. *Cryptosporidium parvum* (*C. parvum*), a protozoan pathogen that causes neonatal calf diarrhea, may impair or alter AA homeostasis since it relies exclusively on host AA to replicate [2]. Therefore, host-pathogen competition for specific AAs may result in significant metabolic alterations in the host. This study aimed to investigate how C.parvum infection influences the AA content of calves' jejunal tissue using targeted metabolomics analysis.

Methods: On day 1 postnatal, Holstein-Friesian calves were orally administrated with one of the following treatments: 1) pure water (CTRL, n = 5), or 2) $2x10^7$ *C. parvum* oocytes as an infection group (INF, n = 5). The experimental calves were fed with 3 l pooled colostrum after birth, thereafter 3 x 2 l milk replacer daily. On day 8 postnatal, jejunum samples were collected from the slaughtered calves. The samples were then processed and analyzed using the targeted metabolomics MxP Quant 500 kit (BIOCRATES LifeSciences AG, Innsbruck, Austria). The fully automated assay combined flow injection and liquid chromatography tandem mass spectrometry (LC-MS/MS). MS analyses were performed on an AB SCIEX 5500 QTrap mass spectrometer (AB SCIEX, Darmstadt, Germany). For peak identification, the Biocrates MeterDQ software was used; metabolite peak areas (as an indication of concentration) were analyzed using Metaboanalyst version 5.0 using Pareto scaling and *t*-Test analysis. AA concentration was reported as fold change (FC).

Results: Targeted metabolite profiling identified 250 metabolites including all 20 AAs in the calves' jejunum. Among the AAs, seven were significantly (p-value < 0.05) different in the infected calves including increased concentrations of tyrosine (Tyr, log2(FC) = 3.80) and arginine (Arg, log2(FC) = 3.76) as well as decreased concentrations of methionine (Met, log2(FC) = -1.91), tryptophan (Trp, log2(FC) = -1.45), alanine (Ala, log2(FC) = -1.49), histidine (His, log2(FC) = -1.31), and cysteine (Cys, log2(FC) = -1.66). The jejunum concentrations of the other AAs were not affected by infection.

Conclusions: Metabolomics results revealed that *C. parvum* infection markedly influenced the jejunum's AA content, which includes both essential (His, Met, Trp, Arg) and non-essential (Tyr, Cys, Ala) AA for neonatal claves. These changes may partially be due to *C. parvum*'s scavenging of epithelial AA, but also might be caused by induction of the calves' inflammatory and immune responses. It is yet unclear whether these local changes are directly reflected by or correlated with the systemic AA metabolic pool. Future studies are needed to investigate the relationship between altered AA homeostasis by infection and in healthy calves.

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83.

Tryptophan metabolism in jejunum mucosa during neonatal calf diarrhea

Tryptophanstoffwechsel in der Dünndarmmukosa bei neonatalem Kälberdurchfall

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The protozoan parasite *Cryptosporidium parvum* (*C. parvum*), a primary cause of neonatal calf diarrhea (NCD), may alter the host's metabolic homeostasis by scavenging amino acids (AA) from the infected epithelium. Tryptophan (Trp) is an essential AA that plays a role in various physiological processes, including metabolism, inflammation, and immune responses, as well as maintaining the gut barrier integrity [1]. In addition, Trp-derived metabolites act as ligand activators for transcription factor aryl hydrocarbon receptor (AhR) as well as G protein-coupled receptors (GPCRs), thereby modulating intestinal inflammatory signaling pathways [2,3]. Nevertheless, little is known about competition between *C. parvum* and host for Trp and how it may affect the host metabolic and immune homeostasis. This study aimed to investigate the effect of *C. parvum* infection on calves' intestinal mucosa focusing on Trp metabolism pathways using a next-generation sequencing (NGS). This Omics-based study is conducted without preconceived hypotheses to gain a deeper understanding of Trp metabolism in jejunum mucosa during *C. parvum* infection.

Methods: Holstein-Friesian calves were randomly assigned to one of two treatments on day 1 postnatal: Oral administration of 2x107*C. parvum* oocytes (infected, n = 5), or pure water as a control (CTRL, n = 5). The feeding regime was identical in all animals, consisting of 3 l pooled colostrum after birth followed by 3 x 2 l milk replacer daily. Jejunum tissue and mucosa samples were collected from slaughtered calves on day 8 postnatal. Tissue samples were used for targeted metabolomics (AA) using MxP Quant 500 kit (BIOCRATES LifeSciences AG, Innsbruck, Austria). Trp concentration in jejunum was reported as fold change (FC) between different groups (infected/CTRL). Total RNA was extracted from mucosa samples and sequenced using the Illumina HiSeq 2500 (San Diego, USA). The raw reads were processed and compared with the bovine reference genome (ARS-UCD 1.2). DESeq2 was used for the statistical analysis and differentially expressed genes (DEG) were defined as those with a false discovery rate (FDR adjusted p-value) < 0.05.

Results: Our preliminary metabolomics data indicated that Trp (log2(FC) = -1.45) and serotonin (log2(FC) = -2.26) concentrations were lower but kynurenine (Kyn) tended to be higher (log2(FC) = 0.25) in the jejunum mucosa of the infected calves. More than 900 DEGs were identified in the calves' jejunum mucosa, of which a number were related to Trp metabolism pathways including the de novo NAD^+ biosynthetic process through the kynurenine (Kyn) pathway. In particular, the expression of indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1), the first and rate limiting enzyme which catabolizes the reaction of Trp to N-formylkynurenine (NFK), was increased in infected calves. However, the downstream genes involved in the conversion of NFK to NAD, such as kynurenine 3-monooxygenase (KMO) and 3-hydroxyanthranilic acid dioxygenase (HAAO) were decreased. Moreover, the relative expression of arylformamidase (AFMID), which is an alternative route and catabolizes NFK to Kyn and later to kynurenic acid (KA), was not affected.

Conclusions: The expression of IDO is evoked in response to *C. parvum* infection and, in particular, by the inflammatory response triggered by interferon-gamma. An increased IDO1 expression is a basic negative feedback mechanism to activate the Kyn pathway and resolve inflammation. Accordingly, metabolomics data revealed a decrease in Trp concentration and tendency for increased Kyn in the jejunum mucosa of infected calves. Future studies are needed to investigate the formation of the canonical IDO-KYN-AhR inflammatory signaling pathway, which in turn interacts with major signaling pathways such as nuclear factor kappa B (NF-κB) to regulate the production of inflammatory cytokines. Altogether, this study provided a deeper understanding of Trp metabolism and its associated feedback regulatory mechanisms during NCD.

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84.

The effect of thermal treatment of field peas on weaned piglets' performance, apparent praecaecal nutrient digestibility, gut morphology, and immunological traits

Der Einfluss einer thermischen Behandlung von Futtererbsen auf die Leistung, die scheinbare praecaecale Verdaulichkeit, die Darmmorphologie und immunologische Merkmale bei Absetzferkeln

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In peas several allergenic proteins have been found. This study aimed to investigate the effect of thermal treatment on peas fed to weaned piglets with special regards to their effects on the gastrointestinal immune system.

Methods: Piglets (n = 48), were weaned with 35 d of life and fed weaning diets (20 % grounded untreated peas (Pea), steamed at 85°C, 10 min (Pea-S), autoclaved at 110°C, 15 min (Pea-A), soybean meal (Con)). After two weeks 24 male piglets were euthanized and ileal digesta, jejunal and colonic tissues were sampled. AB-PAS staining was used to evaluate the morphology, Direct Red 23 to quantify the number of eosinophils and FlowJo was used to evaluate flow cytometric analyses. For statistical analysis a univariate ANOVA was used followed by post hoc Tukey-Test ($P \leq 0.05$).

Results: Average daily feed intake between piglets fed Pea-A diet compared to Con diet ($P = 0.027$) increased significantly. Apparent praecaecal digestibility (pcd) of lysine ($P = 0.044$), methionine ($P = 0.045$), aspartic acid ($P = 0.033$) was higher in pigs fed Pea-A compared to Pea diet. Apparent pcd of phosphorus (P) was higher in piglets fed Pea-A compared to Pea diet ($P = 0.035$). Crypt depth in colon of piglets fed Pea-S diet was significantly lower compared to all other feeding groups ($P < 0.05$). Number of eosinophils in lamina propria of jejunal tissue was significantly lower in piglets fed Pea-A diet compared to piglets fed Con or Pea ($P < 0.001$) diets. Percentage of jejunal CD4+ intraepithelial lymphocytes were significantly lower in piglets fed Pea ($P = 0.029$) and Pea-A ($P = 0.014$) diets compared to piglets fed Con diet.

Conclusions: Results demonstrate an effect of thermal treatment on apparent pcd of amino acids and P as well as an effect of field peas on immunological parameters in jejunal tissue.

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85.

The effect of feeding dried industrial hemp leaves on performance traits and the antioxidant capacity of dairy cows

Der Einfluss der Fütterung von getrockneten Nutzhanfblättern auf die Milchleistung und antioxidative Kapazität von Milchkühen

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Hemp leaves are rich in crude fibre and crude protein [1], and their use as feed does not compete with human nutrition as soya products do, for example. Hemp leaves also contain numerous bioactive secondary plant substances, such as cannabinoids or phenols [2, 3], which possess antioxidative properties known to be important to animal health. However, it is yet unknown if feeding hemp leaves improves in the antioxidant capacity (AC) of dairy cows. The objective of this study was to investigate the effect of exchanging soybean extraction meal against industrial hemp leaves in a total mixed ration (TMR) on performance traits, AC, lipid peroxidation, and plasma amino acid concentration in dairy cows.

Methods: In a cross-over trial, 12 dairy cows in first lactation (> 100 days in milk) were fed *ad libitum* two isoenergetic and isonitrogenous diets. The experimental design consisted of 3 blocks of 4 animals each. In period 1, cows received a TMR supplemented with 7.4% (on the dry matter basis) dried industrial hemp leaves (HEMP) of the variety „Santhica 27" or a TMR containing 3.5 % soy bean extraction meal (CON) for 3 weeks. In the subsequent 2-week washout period, both groups were fed a hemp- and soy-free TMR. In period 2, cows received the opposite diet for further 3 weeks. Daily feed intake and milk yield were measured and milk constitutes analysed by infrared spectroscopy. Blood samples were taken on day 0, 7 and 14, and rumen fluid was collected on day 14. The AC was analysed in TMR, plasma (day 0, 7, 14), rumen fluid, and whey (pooled sample of day 11 evening milking and 12 morning milking), using a ferric ion reducing antioxidant power (FRAP) assay. In addition, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay was used to assess AC in TMR and plasma. Plasma lipid peroxidation was measured using the thiobarbituric acid reactive substances (TBARS). Amino acid concentration in plasma obtained on day 14 was analysed using high-performance liquid chromatography. The data were analysed with a linear mixed model in R (4.3.1). The models included group (HEMP, CON), sequence, period and block as fixed effects, and animal as random, except for ABTS and FRAP in TMR, which was analysed using a linear model, including group and block as effects. Model for the analysis of plasma FRAP and ABTS included time as additional fixed effect.

Results: Cows of the HEMP group ingested 1.7 kg/d less dry matter and produced 1.4 kg/d less energy-corrected milk yield compared to the CON group ($P < 0.05$). Milk composition was comparable between groups ($P > 0.1$). The AC of the HEMP diet was similar to the CON diet when determined by the FRAP assay, but when measured by the ABTS assay, the HEMP TMR tended to have a higher antioxidant capacity compared to the CON TMR ($P = 0.062$). In plasma, the AC was comparable between groups, no matter if determined by FRAP or ABTS ($P > 0.05$). In addition, the AC of rumen fluid and whey, as well as plasma TBARS concentrations did not differ between groups ($P > 0.1$). Yet, cows of the HEMP group had 41.3% greater plasma anserine concentration ($P < 0.001$), 9.6% lower π-methylhistidine concentration ($P < 0.002$), and tended to have 12.3% lower isoleucine concentration compared to CON cows ($P = 0.056$).

Conclusions: The inclusion of 7.4% industrial hemp leaves in the TMR of dairy cows affected the feed intake and the productivity of cows negatively, presumably due to the unaccustomed flavour. After accustoming to the flavour, hemp leaves could, at least partially, replace soybean extraction meal in a diet for dairy cows. Although the HEMP diet possessed a slightly higher antioxidant capacity, its ingestion did not alter AC or lipid peroxidation in plasma, despite an increase in the concentration of anserine, a free radical scavenger. The increase in plasma anserine concentration occurred on the expense of π-methylhistidine concentration, but if protein catabolism is affected by HEMP feeding needs to be investigated in future studies.

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86.

Changes in intracellular carnitine levels and gene expression in bovine blood cells during lipopolysaccharide-induced inflammation and dietary L-carnitine supplementation

Veränderungen der intrazellulären Carnitin Konzentration und Genexpression von bovinen Blutzellen während einer Lipopolysaccharid-induzierten Entzündung und einer L-Carnitin Supplementierung

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L-carnitine plays a pivotal role in energy metabolism, particularly in the transport of fatty acids into the mitochondrial matrix. Consequently, it may influence the immune function of peripheral blood mononuclear cells (PBMC), especially under inflammatory conditions. This study aimed to investigate the effects of L-carnitine supplementation on intracellular carnitine levels and the expression of specific genes in bovine PBMC during energy-demanding lipopolysaccharide (LPS)-induced inflammation.

Methods: This study was conducted as part of a trial that lasted from day 42 prior to the expected calving to day 128 *post partum (pp)* [1], referring to day 143 before until 14 days after the immune challenge. A total of 53 pluriparous German Holstein cows were assigned to two groups: an L-carnitine supplemented group (CAR; n = 27) and a control group (CON; n = 26). The two groups were fed a partial mixed ration comprising 50% concentrate feed delivered via electronic feeding stations and 50% roughage (70% maize silage, 30% grass silage) provided in feed-weigh troughs. CAR was administered a rumen-protected L-carnitine product (Carneon 20 Rumin-Pro, Kaesler Nutrition GmbH, Cuxhaven, Germany) at a dosage of 125 g per cow and day, amounting to a daily intake of 25 g L-carnitine per cow. CON was obtained a matching product with a similar fat content (BergaFat F-100 HP, Berg + Schmidt GmbH & Co. KG, Hamburg, Germany). Water was offered *ad libitum*. On day 111 pp, each cow received an intravenous injection of LPS (0.5 µg/kg body weight, *E. coli*, Serotype O111:B4, Sigma Aldrich, St. Louis, Missouri, USA). Heparinized blood was collected at -143 d, -11 d, 24 h, and 14 d relative to LPS injection and PBMC were isolated via density gradient centrifugation. Intracellular carnitine and its derivatives were analyzed in PBMC using tandem mass spectrometry (3200 Q Trap, AB Sciex, Framingham, Massachusetts USA), with protein content serving as normalization factor. Furthermore, RT-qPCR (Fluidigm, BioMark HD, San Francisco, California, USA) was performed, and the data were processed with R Studio (version 2023.12.1+402, R version 4.3.2) to generate calibrated normalized relative quantities (CNRQ) according to Hellemans et al. [2] to investigate changes in gene expression of PBMC. Statistics were calculated using the MIXED procedure of SAS (9.4) including time, group and their interaction as fixed factors.

Results: The influence of L-carnitine supplementation on intracellular carnitine and γ-butyrobetaine differed significantly over time ($p_{group*time} < 0.001$). In contrast, N-trimethyllysine was not affected by L-carnitine. However, the last three time points showed significantly decreased concentrations in comparison to the initial time point (ptime < 0.001). The CNRQ of solute carrier family 25 member 20 (SLC25A20) was significantly affected by the interaction of group and time ($p_{group*time} = 0.036$). CON showed a significant increase in expression 24 h after LPS injection, while CNRQ in CAR remained at the same level. Additionally, expression of cytochrome c oxidase subunit 4I1 (COX4I1) varied differently with L-carnitine supplementation and LPS administration ($p_{group*time} = 0.017$). The LPS injection led to a significant decrease in CNRQ values at 24 h in CAR, while CON maintained the baseline level.

Conclusions: In conclusion, daily supplementation of 25 g L-carnitine per cow resulted in significantly higher intracellular carnitine and γ-butyrobetaine concentrations in PBMC, indicating an effective absorption and distribution of L-carnitine in dairy cows. The up-regulation of SLC25A20, which encodes carnitine-acylcarnitine translocase, in CON suggests an increased demand for acylcarnitine transport into the mitochondrial matrix during systemic inflammation when L-carnitine is not supplemented.

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87.

Effect of a subclinical ketosis challenge on biomarkers of energy metabolism and liver function and their variability in dairy cows during the transition period

Auswirkungen einer induzierten subklinischen Ketose auf Biomarker des Energiestoffwechsels und der Leberfunktion und deren Variabilität bei Milchkühen in der Transitphase

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Subclinical ketosis (SCK) is the most prevalent metabolic disorder in early-lactating dairy cows, caused by excessive adipose tissue mobilization, leading to increased production of non-esterified fatty acids (NEFA). Prolonged high NEFA levels can impair liver function, though individual responses vary largely [1]. This study aimed to examine the effect of an induced SCK challenge on energy metabolism and liver function biomarkers, and their variability in dairy cows during the transition period in a forage-based feeding system.

Methods: Forty-two d before the predicted calving date, 16 multiparous Holstein and Red Holstein cows were assigned to one of two treatments based on body condition score (BCS). Cows with a BCS of ≤ 2.75 served as control group, while those with a BCS of > 2.75 underwent a SCK challenge based on the animal model of Schulz et al. [2]: Antepartum(ap), the control group was fed an *ad libitum* partial mixed ration (PMR) according to recommendations for transition cows (5.0 MJ NEL / kg DM). SCK cows were fed an energy-richer PMR (5.9 MJ NEL / kg DM), plus 5.4 kg/d of concentrate (8.4 MJ NEL / kg DM). Postpartum(pp), all cows were fed a PMR with control cows receiving higher quantities of energy concentrate (2.4 kg/d staggered increase to 6.4 kg/d after d 14) than SCK cows (1.8 kg/d staggered increase to 5.4 kg/d after d 21). Feed intake, body weight and pp milk yield were recorded daily. Postpartum, milk and blood samples (vena jugularis) were taken daily. Milk was analysed for fat, protein, and lactose and serum for concentrations of beta-hydroxybutyrate (BHB), NEFA, glucose, creatinine, aspartate transaminase (ASAT), alanine transaminase (ALAT) and gamma-glutamyltransferase (GGT). Energy was expressed in MJ NEL, following current Swiss practice. Statistical analyses used linear mixed models with treatment, week and their interaction as fixed factor (random factor: animal) and coefficients of variation (CV) in the R environment.

Results: Total dry mater intake (DMI) increased over time ($P<0.001$), depending on the treatment, with greater pp DMI for CON (22.8 ± 2.1 kg/d) than SCK (19.9 ± 2.9 kg/d) cows (interaction: $P<0.001$). Milk yield was similar for CON and SCK cows, so were protein (and lactose contents (all $P \geq 0.15$). Fat content was lower ($P=0.04$) for CON (4.2 %) than SCK (4.5 %) cows. Over the weeks pp, milk yield and lactose increased and protein decreased (all $P < 0.001$). The calculated EB was ap lower and pp higher (both $P<0.01$) for CON (10.4 (ap) and -7.7 (pp) MJ NEL) than SCK cows (34.7 (ap) and -41.2 (pp) MJ NEL). BHB levels increased over time ($P=0.004$), depending on the treatment (interaction: $P = 0.003$), with similar values in weeks 1 and 2 and lower values for CON than SCK cows in weeks 3 and 4 pp. NEFA levels were lower ($P<0.001$) for CON (0.13 mmol/L) than SCK (0.33 mmol/L) cows and decreased over time ($P<0.001$). Glucose and creatinine levels fluctuated and decreased over time, respectively (both $P\leq0.018$). Levels of hepatic enzymes were in the reference ranges for all cows. ASAT and GGT levels were similar (both $P\geq0.44$), with GGT increasing over time ($P<0.001$). ALAT levels were influenced by time depending on the week pp, with greater levels for CON than SCK cows in W4 pp (interaction: $P<0.001$). Seven out of eight SCK cows and five of eight CON cows had serum BHB levels ≥ 1.2 mmol/l on at least one day pp, with a lower number of days for CON (1-7 d) than SCK (5-24 d) cows. Serum concentration of all analytes had lower CV for CON than SCK cows (e.g., BHB: 29% (CON) vs 52% (SCK); NEFA: 48% (CON) vs 82% (SCK)), while CV of DMI (16%) and milk yield (17%) were markedly lower and similar for CON and SCK cows.

Conclusions: The animal model induced SCK in dairy cows in a forage-based feeding system without harming liver health. The great variation in energy metabolism biomarkers among SCK cows confirms previous findings [1]. Further research should investigate the underlying reasons of this variability.

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88.

Preliminary data on the interaction of health status and diet zinc in piglets from Bavarian producers

Vorläufige Daten zur Interaktion von Gesundheitsstatus und Zink im Futter bei Ferkeln bayerischer Erzeugerbetriebe

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Since 2019, the Bavarian Animal Health Service (TGD) regularly registers farms with clinical events of zinc deficiency. Causes were often an interaction between inhomogenous feed batches and high iron background in commercial premixes (6000-8000 mg/kg premix). This study presents first data on the effects of veterinary intervention on such farms.

Methods: Eleven piglet producers in southern Bavaria were surveyed by the TGD during regular herd management. Farms had ongoing cases of weaned piglets with zinc deficiency symptoms like acrodermatitis and poor weight gain. Veterinary intervention included improving feed homogeneity through better on-farm mixing and changing premix on all farms. Additionally, four of the eleven farms received extra Zn supplementation to raise dietary Zn to the legal limit (150 mg/kg diet; 50:50 ZnO and $Zn_5(OH)_8Cl_2$ added Zn). At a follow-up examination after 14-21 days, five previously Zn-deficient animals and five control animals per farm were weighed, rectal temperatures taken, and blood samples collected. Serum levels of Zn, Fe, Cu, C-reactive protein (CRP), interleukin 6 (IL6), and Mycoplasma hyopneumoniae (Mhyo) antibody titers post vaccination were measured. Data normality was checked with quantile-quantile plots and analyzed using mixed models for fixed effects of „health status“ and „zinc supplementation“ with herd as random factor.

Results: Zn-deficient animals had lower live weight compared to controls (21.9 Cl[19.8, 24.1] vs. 23.2 Cl[21.0, 25.4] kg; P = 0.005). Zinc addition reduced the live weight of control piglets by 0.9 kg, while Zn-deficient animals improved by 0.7 kg (P = 0.005). Serum Fe tended to decrease in Zn-deficient piglets (83.5 Cl[73.5, 93.5] µg/dL) vs. controls (92.4 Cl[82.4, 102] µg/dL; P = 0.08); dietary Zn increased Fe from 70.3 to 96.7 µg/dL (P = 0.01). Serum Zn showed no distinct effects of health status or diet Zn but a significant statistical interaction between both variables was evident (P = 0.003); Zn-supplemented controls showed decreased levels, while Zn-deficient animals with extra Zn showed increased levels. Circulatory Cu was higher in Zn-deficient animals (155 Cl[142, 168] vs. 143 Cl[139, 156] µg/dL; P = 0.03). Serum CRP was elevated in Zn-deficient animals (103 Cl[38.8, 166] vs. 37.0 Cl[–26.7, 101] µg/mL; P = 0.002); both healthy and Zn-deficient animals had lower CRP with extra Zn (P = 0.005). Serum IL6 was higher in animals receiving extra Zn regardless of health status (19,239 Cl[9,834, 28,645] vs. 86.6 Cl[–7,002, 7,175] pg/mL; P = 0.002). Zn- supplementation increased Mhyo antibody titers in control and Zn-deficient animals (P=0.0006), with the effect being most pronounced in the control animals (P = 0.002).

Conclusions: The trace metal profiles (Zn, Fe, Cu) in the sera of Zn-deficient and control animals suggested ongoing acute-phase reactions [1], consistent with the CRP response and expected in animals with prior clinical Zn deficiency [2]. Interestingly, additional Zn supply up to the legal limit improved live weight in Zn-deficient animals but impaired weight of controls, indicating detrimental effects of excess Zn in the latter. This suggests that increasing dietary Zn is suitable only as a targeted measure in Zn-deficient animals, not at the herd level. This finding has implications for interpreting IL6 data: the rise in circulatory IL6 due to Zn addition may indicate restoration of impaired synthesis in Zn-deficient animals or reflect a pro-inflammatory stimulus from excess Zn in controls, aligning with the live weight data. The significant effect of Zn addition on Mhyo antibody titers particularly in control animals is interesting and warrants further research. Whether the reduced circulatory Zn levels were in any case the cause of health issues or sometimes just a symptom of ongoing acute-phase reactions has yet to be determined.

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89.

Evaluation of the effects of soluble and insoluble fibre on the intestinal microbiota of growing pigs inoculated with *Brachyspira hyodysenteriae*

Beurteilung der Effekte löslicher und unlöslicher Faser auf die intestinale Mikrobiota von wachsenden, mit Brachyspira hyodysenteriae inokulierten Schweinen

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The composition of the pig‘s diet and its influence on the microbial composition in the colon can affect the timing and severity of swine dysentery [1], a disease that can be caused by the colonization of the colon with pathogenic *Brachyspira (B.) hyodysenteriae*. Nevertheless, contradictory results have been found between different research groups and it is suspected that the different structures of carbohydrates and fibers within the experimental diets influence the microbial activity and composition to different degrees [2].

The aim of the present study was to investigate the effects of varying levels of soluble and insoluble fibre on the intestinal microbiota of growing pigs inoculated or not with *Brachyspira hyodysenteriae*.

Methods: A total of 48 growing pigs (26.9 ± 2.5 kg) were allocated into six blocks and fed one of four diets for 6 weeks: low fiber diet based on wheat and soybean meal (LF), high fiber diet based on barley, barley hulls and soybean meal (HF), high soluble fiber diet based on wheat, sugar beet pulp, potato pulp, pectin residue and soybean meal (HS), and high insoluble fiber diet based on wheat, seed residue, pea hulls and brewer’s spent grain and soybean meal (HI). The diets were formulated to meet the requirements of the Danish minimum recommendations for essential nutrients [3]. After two weeks, half of the pigs (n = 24) were inoculated with *B. hyodysenteriae* orally daily for three consecutive days (dosage: 5 x 10^{10} cfuper pig). The strain was isolated from pigs in a commercial Danish farm showing clinical symptoms of swine dysentery. At week six, four weeks after experimental inoculation, pigs were euthanized to assess microbiota composition in freeze-dried samples obtained from the digesta of the small intestine, caecum and colon via 16S rRNA gene sequencing. R (version 4.1.2) was used for microbiota data analyses. Alpha diversity indices were calculated with the R-package ‘phyloseq’ (version 1.36.0) to evaluate bacterial richness and diversity. Microbiota composition of samples were assessed for variation in relation to the ‘Inoculation status’ and ‘Diet’ by a permutational multivariate analysis of variance (PERMANOVA) using the Bray-Curtis distance.

Results: At week six, average body mass of non-inoculated pigs (57.6 kg) differed significantly (SEM: 1.7; $p = 0.001$) from that of *B. hyodysenteriae*-inoculated pigs (43.7 kg). The dietary composition had no effect on the body mass of the pigs (SEM: 2.4; $p = 0.761$). *B. hyodysenteriae* inoculation did not influence the microbiota richness and diversity, but altered significantly total community structure and composition of small intestinal ($R^2 = 0.060$, $p = 0.023$) and colonic ($R^2 = 0.068$, $p = 0.013$) microbiota of pigs. The dietary composition influenced significantly caecal microbiota richness of non-inoculated pigs (HF: 249^{ab} ±34.5, HI: 232^{ab} ±121, HS: 320^{a} ±65.4, LF: 182^{b} ±41.1, $p = 0.031$), but not of *B. hyodysenteriae*-inoculated pigs (HF: 258 ±126, HI: 216 ±130, HS: 324 ±92.6, LF: 256 ±143; $p = 0.529$). In addition, microbiota composition of samples taken from *B. hyodysenteriae*-inoculated pigs were not influenced significantly by the factor ‘Diet’. In contrast, PERMANOVA exposed the factor ‘Diet’ to be a significant source of variation of microbiota composition in samples taken from the caecum of non-inoculated pigs ($R^2 = 0.235$, $p = 0.016$). A trend could also be observed for the samples of the small intestine ($R^2 = 0.233$, $p = 0.052$).

Conclusions: The findings suggest that expected effects of a dietary measure on intestinal microbiota might be different under the influence of an intestinal disease.

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90.

Microbial fermentation products along the intestinal tract of horses fed starch containing vs forage only diets

Mikrobielle Fermentationsprodukte im Darmchymus von Pferden nach Fütterung stärkehaltiger Rationen im Vergleich zu alleiniger Grobfuttergabe

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Although horses are adapted to a low-energy and high-fibre feed, most common rations contain high-energy, low-fibre concentrates. Starchy diets affect microbial composition (MC) and fermentation products (MFP) in the horses' gut, although their significance is not yet fully understood. For example, even small amounts of oat starch, despite its known high praecaecal digestibility, lead to measurable changes in the faecal MC [1]. The objective was to investigate the impact of common horse diets (pasture or hay *ad libitum*, the latter without or with different oat grain dosages) on the dry matter (DM) content and concentration of MFP in digesta alongside the horses' intestine. The hypothesis was that each diet leads to its own patterns.

Methods: 23 horses (age 11 ± 6.0 years; body weight [BWT] 471 ± 63.4 kg; body condition score 5.1/9 ± 0.67; cresty neck score 1.0/5 ± 0.70) were allocated to four rations: hay ad lib. (HAY; n = 8), hay ad lib. plus oats at 1 g starch/kg BWT/meal (OS1; n = 6), hay ad lib. plus oats at 2 g starch/kg BWT/meal (OS2; n = 5) or 24 h/d pasture (PST; n = 4). Oats was offered once a day as whole grains. Horses had free access to a salt lick and tap water. Stabled horses had straw as bedding. After at least 34 days, feeding periods ended with euthanasia, dissection and sampling. Digesta samples were taken from the following intestinal sections: jejunum, caecum, colon ventrale dextrum, colon ventrale sinistrum, pelvic flexure, colon dorsale sinistrum, colon dorsale dextrum, colon transversum and rectum. Samples were stored at - 20 °C until dry matter (DM) was determined and pH, short chain fatty acids (SCFA) and ammonia were detected in digesta-innate water. The fixed effects ration and intestinal section and their interactions were assessed by procedure MIXED (SAS 9.4) with horse as random effect. Differences with $P < 0.05$ were considered significant.

Results: Horses fed OS1 had overall higher digesta DM contents than horses fed PST ($P = 0.038$) or HAY ($P = 0.010$). This might be explained by reduced salivation with concentrate feeding [2], lower water binding capacity and ration specific gastro-enteral secretion rates, respectively. PST horses showed highest concentrations of iso-fatty acids in the digesta from the pelvic flexure to the colon transversum ($P < 0.05$). Since iso-fatty acids are associated with the microbial breakdown of amino acids [3], it can be assumed that intestinal protein metabolism is increased in PST. Ammonia, pH and SCFA other than iso-fatty acids did not differ among rations ($P > 0.05$).

Conclusions: Under feeding conditions as in the present study, higher concentrations of iso-fatty acids in the intestinal digesta indicate higher microbial protein turnover in horses on pasture whereas the difference between hay alone and hay plus oats is rather small.

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91.

Digestion of carbohydrates in the stomach of horses on pasture, or with feeding of hay, or hay and oats

Kohlenhydratverdauung im Magen von Pferden auf der Weide, bei Fütterung von Heu, oder Heu und Hafer

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Carbohydrate digestion in the stomach is facilitated by commensal microbes, naturally not adapted to diets rich in starch. Excess supply of horses with soluble carbohydrates, especially starch, poses a risk for dysbiosis and gastric disease. The objective was to analyse how starch, mono- and disaccharides, fructans, and fibres disappear from gastric digesta in relation to acid-insoluble ash (AIA) as marker.

Methods: 24 horses (13 m/11 f), 11 ± 6.0 years, 471 ± 63.4 kg body weight (BWT), were allocated to the following rations: 24 h/d pasture (PST, n = 4), hay ad lib. (HAY, n = 8), hay ad lib. and oats (1 g starch/kg BWT/meal, OS1, n = 6), and hay ad lib. and oats (2 g starch/kg BWT/meal, OS2, n = 6). One horse was excluded from the analyses. The oat meal was offered once a day. Horses had free access to water and salt, and in the stable, to straw bedding and a paddock. Rations were fed at least for 34 d. The horses were euthanised to collect digesta from the Pars nonglandularis (PNG) and Pars glandularis (PG). Starch, mono- and disaccharides, fructans, detergent fibres, and AIA were analysed in feed bulk samples and digesta, and the proportion Q analyte to marker was calculated. Statistical analysis was performed with SAS 9.4 (SAS Institute Inc.) using the MIXED procedure.

Results: The intake within 2 h before sedation was as follows (g): dry matter: 1,891 ± 233.1 (PST), 1,068 ± 494.6 (HAY), 2,267 ± 636.8 (OS1), 3,587 ± 682.4 (OS2); oat starch: 497 ± 121 (OS1), 1,046 ± 209.6 (OS2); glucose: 14.1 ± 1.75 (PST), 14.2 ± 7.88 (HAY), 22.8 ± 16.5 (OS1), 26.2 ± 11.6 (OS2); fructose: 36.9 ± 4.55 (PST), 47.5 ± 34.0 (HAY), 52.3 ± 28.8 (OS1), 51.4 ± 8.39 (OS2); sucrose: 11.4 ± 1.41 (PST), 11.6 ± 13.6 (HAY), 17.6 ± 5.35 (OS1), 37.8 ± 14.0 (OS2); fructans: 82.7 ± 10.2 (PST), 37.3 ± 32.4 (HAY), 46.5 ± 28.0 (OS1), 77.7 ± 63.1 (OS2); hemicellulose: 493 ± 60.8 (PST), 279 ± 128 (HAY), 477 ± 147 (OS1), 716 ± 130 (OS2); cellulose: 631 ± 77.8 (PST), 351 ± 161 (HAY), 527 ± 191 (OS1), 707 ± 187 (OS2). Up to 337 and 414 g oat starch/kg dry matter was detected in digesta in OS1 and OS2, respectively. Q-starch decreased from PNG to PG ($P < 0.05$), by 57% in OS1 and 42% in OS2. Q-glucose, Q-fructose, and Q-sucrose were reduced from PNG to PG. In PST and HAY, sucrose was completely cleared ($P < 0.05$). Q-fructans was reduced from feed to PNG by 84% (PST, $P < 0.05$) and 55% (HAY). In PST, it increased from PNG to PG. Fructans did not disappear with the high-starch diet (OS2). Reduction of Q-fibres was mainly in PST and HAY ($P < 0.05$).

Conclusions: Disappearance of starch, mono- and dimeric sugars, fructans, and fibres was observed in the stomach, as a result of acid hydrolysis, fermentation, or rapid flux with liquids into the small intestines, and was specific for the ration type to which the horses were adapted. The results indicate that recurrently high proportions of starch in gastric digesta impair the digestion of fructans. This could result from differently adapted microbial communities (fructan depolymerisation by microbial enzymes) and/or lesser hydrochloric acid penetrating the digesta in the grain meals (acid hydrolysis of fructans).

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92.

How does β-alanine influence total gas and methane production in *in vitro* rumen fermentation

Wie beeinflusst β-Alanin die Gesamtgas- und Methanproduktion in der in vitro Pansenfermentation

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β-Alanine (β-Ala) is a non-proteinogenic amino acid and the only naturally-occurring β-amino acid. A recent study showed that supplementation with β-Ala modified the rumen bacterial community and improved the digestibility of dry matter, organic matter and crude protein and the nitrogen retention in beef steers [1]. However, it is unknown if β-Ala has any impacts on rumen total gas and methane (CH_4) production.

Methods: Three adult Angus steers, fitted with rumen cannulas and fed with a total mixed ration, were used as the donors of rumen fluid. The *in vitro* rumen gas production technique of Menke et al. (1988)[2] was used for incubation. Four levels of β-Ala (purity ≥ 99.0%), i.e., 0, 5, 10 and 20 mg, were added to 0.200 g air-dried feed mixture composed of 50.0% corn silage, 26.5% corn grain, 13.0% soybean meal, 9.5% wheat bran, 0.5% sodium bicarbonate and 0.5% sodium chloride on dry matter (DM) basis as treatments. Each treatment contained 7 replicates and the samples were incubated at 39 °C in an incubator for 48 h. The incubation was terminated at 48 h. The total gas production of each treatment was recorded and the gas was sampled. The CH_4 and CO_2 of the gas samples and the volatile fatty acids (VFA) of incubation liquids were analyzed on a gas chromatograph. The linear effects of increasing levels of β-Ala on different fermentation parameters were analysed using the CONTRAST procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC, USA).

Results: Adding β-Ala at 0, 5, 10 and 20 mg into 0.2 g air-dried feed mixture linearly decreased the 48 h-total gas production from 251.0 ± 4.0 to 227.2 ± 5.0, 232.7 ± 5.8 and 235.2 ± 5.7 mL/g DM ($P = 0.006$), decreased the 48 h-CH_4 production from 36.0 ± 4.4 to 33.7 ± 5.4, 31.1 ± 2.6 and 30.0 ± 3.8 mL/g DM ($P = 0.026$), and decreased the 48-h CO_2 production from 151.4 ± 5.1 to 140.8 ± 5.5, 137.0 ± 4.7 and 127.5 ± 4.7 mL/g DM ($P < 0.001$), respectively. Adding β-Ala at 0, 5, 10 and 20 mg into 0.2 g feed mixture (air-dry basis) tended to decrease the 48 h-total VFA concentration of incubation liquids from 101.1 ± 10.4 to 96.4 ± 9.6, 98.9 ± 6.1 and 92.4 ± 8.2 mmol/L ($P = 0.096$) and significantly decreased the 48-h acetate/propionate ratio from 2.37 ± 0.03 to 2.35 ± 0.05, 2.33 ± 0.04 and 2.33 ± 0.05 ($P = 0.020$), respectively.

Conclusions: β-Ala decreased the acetate/propionate ratio and the total gas and CH_4 production in *in vitro* rumen fermentation. *In vivo* experiments are necessary to verify the results and to investigate the impact mechanisms.

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93.

The effect of different proportions of soybean oil and concentrate in the ration on ruminal protozoa, volatile fatty acid concentrations and methane emissions in dairy cows

Der Einfluss von unterschiedlichen Sojaöl- und Kraftfutteranteilen in der Ration auf die ruminalen Protozoen- und flüchtige Fettsäuren Konzentrationen, sowie die Methanemissionen bei Milchkühen

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Ruminal protozoa are known to produce volatile fatty acids (VFA) [1], releasing hydrogen, which is then mainly converted into methane (CH_4) by methanogens. It is known that a high proportion of fats in the ration of dairy cows can influence microbial fermentation [2]. The objective of this study is to investigate whether the proportions of soybean oil and concentrate in the diet of dairy cows affect the ruminal concentration of protozoa and VFA as well as the emission of methane.

Methods: Data was obtained from an ongoing project, with 48 lactating German Holstein cows, allocated equally into four groups according to a 2x2 factorial design. The factors were soybean oil content (low content of 0.25% soybean oil in dry matter (DM), SL; high content of 2.5% soybean oil in DM, SH) and concentrate content (low content of 30% concentrate in DM, LC; high content of 55% concentrate in DM, HC). The trial lasted six weeks, first three weeks representing an adaptation phase and last three weeks the collection phase. Before the actual test phase, a zero sample was taken. Feeding was carried out as partial mixed ration (PMR). Feed intake (kg DM Intake (DMI)) was determined via automatic feed weighing troughs. Concentrate was fed via automatic concentrate feeders and GreenFeed systems (C-Lock Inc., Rapid City, USA). GreenFeed systems were also used to measure ruminal CH_4 emissions. VFA and protozoa in the rumen fluid were determined in weeks 0,4 and 6 from samples, which were collected through oral rumen probe. VFA concentrations were determined by gas chromatography. Protozoa were microscopically differentiated into large and small entodiniomorphs, Isotricha and Dasytricha using a Nageotte counting chamber, quantified and extrapolated to cells per ml. Statistical calculation was performed with R (Version 4.3.2) and R Studio (Version 2023.12.1) using the package "nlme" to create mixed models. The fixed effects included soybean oil content, concentrate content, week of trial and the zero sample values of the respective dependent variable as covariates. A Box-Cox-transformation was carried out for the protozoa concentrations due to their non-normal distribution.

Results: Soybean oil content or the concentrate content had no significant effect on the total protozoa concentration (in cells/ml), except in the case of large entodiniomorphs. Here, a significant effect of concentrate ($p < 0.01$) was shown, with the concentration being higher in LC groups compared to HC groups. Additionally, an effect of the week was observed, with concentrations being lower in week 4 compared to week 6 ($p < 0.01$). The total concentration of VFA did not show significant differences between treatment groups ($p > 0.05$), but total concentrations were lower in sample week 4 compared to week 6 ($p = 0.01$). For individual VFA, the concentrate content had a significant effect on propionate and valerate ($p < 0.01$), with lower concentrations in LC groups compared to HC groups. Levels of acetate trended ($p = 0.058$) to be higher in the LC groups than in the HC groups. Butyrate exhibited a significant effect of the soybean oil content ($p < 0.01$), with higher concentrations found in SH groups compared to SL groups. Ruminal CH_4 production, calculated in grams per cow and day (g/d), was significantly higher in the LC groups compared to the HC groups ($p < 0.01$) In SH compared to SL groups and in LC compared to HC groups, methane yield (g CH_4/kg DMI) was significantly ($p < 0.01$) higher. Feed intake (kg DMI) was significantly higher ($p < 0.05$) in SL than in SH groups and in HC than in the LC groups.

Conclusions: The results of the study indicate that the increased soybean oil proportion of 2.5% in DM does not significantly affect either the total concentration of protozoa and VFA in rumen fluid nor ruminal methane emissions in g/d. However, it does affect the production of butyrate and methane yield and feed intake. Further research is needed to explore whether other factors may contribute to these observed effects. The project „Nemur" was funded by the Federal Ministry of Food and Agriculture as part of the German Climate Protection Programme 2022.

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94.

Influence of feeding *Pleurotus sapidus* fungus fruiting bodieson the performance, the gut microbiome and the transcriptome and the lipid metabolism in the liver of broilers

Einfluss der Fütterung von Pleurotus sapidus-Pilzfruchtkörpern auf die Leistung, das Darmmikrobiom und das Transkriptom und den Lipidmetabolismus der Leber von Broilern

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Despite the need to enhance feed production for monogastric farm animals, this challenge is complicated by the fact that natural resources are becoming increasingly limited and climate protection goals have to be considered. Thus, there is a need for alternative feed sources, which are produced in a resource-efficient and sustainable manner. Valorization of low-value agricultural sidestreams by edible fungi, such as *Pleurotus sapidus* (PSA), has been proposed as a strategy to utilize such sidestreams as a sustainable source of feed for monogastric farm animals, because Pleurotus spp. are capable of degrading a wide array of fiber-rich substrates. In a recent study [1], we have demonstrated that feeding a biotechnologically produced PSA mycelium to broilers does not affect growth performance and nutrient digestibility and causes only weak effects on the cecal microbiota community, the liver transcriptome and the plasma metabolome of broilers. In order to clarify if the effect of the mycelium differs from that of the fruiting bodies, the present study investigated the effect of feeding PSA fruiting bodies on the performance, the gut microbiome and the transcriptome and the lipid metabolism in the liver of broilers.

Methods: 72 male, 1-day-old Cobb 500 broilers were randomly assigned to three different groups and fed three different complete diets containing either 0 (PSA-0), 25 (PSA-25) or 50 (PSA-50) g/kg diet of PSA fruiting bodies, which were included at the expense of wheat, in a three-phase feeding system for 35 days. After killing the animals, blood plasma, liver, digesta from ileum and cecum were collected and stored at -80°C until analysis. The cecal digesta microbial community was analyzed by 16S rRNA-based high throughput sequencing. Cecal digesta concentrations of short-chain fatty acids (SCFA) were analyzed by gas-chromatography flame-ionisation detection. Concentrations of triglycerides (TG) and cholesterol (Chol) in liver lipid extracts were analyzed by enzymatic test kits. Liver transcriptomics was carried out using an Affymetrix chicken GeneChip. Experimental units was n = 6 cages/group for feed intake and feed:gain ratio, n = 6 broilers/group for transcriptomics data, n = 12 broilers/group for biochemical parameters and 16S rRNA-based high throughput sequencing data, and n = 23-24 broilers/group for body weights and body weight gain. Normally and not-normally distributed data were analyzed by one-way ANOVA and Kruskal-Wallis test, respectively.

Results: Body weights at d 35 and body weight gain during the whole period was reduced in groups PSA-50 and PSA-25 compared to group PSA-0 ($P<0.05$), whereas feed intake and feed:gain ratio during the whole period and apparent ileal digestibility of crude protein and crude fat were not different between groups. The cecal microbial α-diversity indicators Chao1 and Richness differed between groups ($P<0.05$), whereas the indicators of microbial β-diversity (Bray-Curtis, Jensen-Shannon-divergence, Jaccard) were not different between groups. Taxonomic analysis revealed a higher abundance of the class Bacilli and the species unknown_Erysipelatoclostridium and a lower abundance of the class Clostridia in group PSA-50 than in group PSA-0 ($P<0.05$). Concentrations of total and the individual SCFA acetic acid and propionic acid in the cecal digesta were lower in group PSA-50 than in group PSA-0 ($P<0.05$). Liver transcriptomics revealed only 8 hepatic transcripts to be expressed differentially between group PSA-50 and group PSA-0 (Fold change >1.5 and <-1.5, $P<0.05$). Hepatic concentrations of TG and Chol were not different between groups.

Conclusions: The study shows that inclusion of PSA fruiting bodies in broiler feed reduces growth performance but has only marginal effects on the cecal gut microbiome and the liver transcriptome and hepatic lipid metabolism of broilers. Based on these results the inclusion of PSA fruiting bodies at the concentrations tested in broiler feed cannot be recommended.

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95.

How does a low carbohydrate diet affect metabolite profiles in wild type and GIPR^{dn} transgenic pigs?

Wie wirkt sich eine kohlenhydratarme Fütterung auf die Metabolitenprofile von Wildtyp- und GIPR^{dn} transgenen Schweinen aus?

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Pigs are widely used as model organisms for human metabolic diseases. The GIPR^{dn} transgenic pigs express a modified receptor for the gastric inhibitory polypeptide (GIP). As a result of the impaired incretin effect, they have a lower beta cell mass, blood insulin levels and thus a reduced glucose tolerance, similar to the human metabolic syndrome. They express so that pancreatic development is missing its main stimulus. They secrete A high fat, low carbohydrate diet with a moderate protein content was fed in order to challenge the pigs´ energy metabolism into potential decompensation of glucose tolerance. Metabolomic approaches were used to identify potential biomarkers of this state.

Methods: Wild type (WT) and GIPR^{dn} transgenic pigs (n=16 each, 6 mo) were randomly allocated in the control group (CON) or the low carb, moderate protein (LCMP) diet group (n=8/group). The LCMP group was fed the LCMP diet (14.1% CF, 13.5% CP, 62.9% CL, 7.0% CA) for 4 wks, then switched to the CON diet (5.4% CF, 18.5% CP, 4.7% CL, 4.6% CA) for another 4 wks. The CON group was fed the CON diet for the complete trial. Feed was allocated to achieve slow and similar body weight increase and avoid fattening. Blood samples were taken at the start (w0), after 4wks (w4) and at the end of the trial (w8). Plasma was obtained in a standardized procedure for metabolomics analysis (AbsoluteIDQTM p180 Kit, biocrates Life Sciences AG). Metabolite measurements were log transformed and normalised using variance stabilisation. Statistical modelling was performed using the limma (linear models for microarray data) package, taking into account the animal as a random factor for time series analyses.

Results: The effect of the genotype was significant for 7 metabolites, which have in part already been described for GIPR^{dn} transgenic pigs and may indicate impaired metabolic control (e.g. phosphatidylcholine (PC) aa C38:1 elevation). There was a significant effect of diet * time on 76 of the 188 metabolites measured. At w4, there was a cluster of lower metabolite concentrations in the LCMP fed pigs for metabolites like spermidine, Gln, Ser & Pro in both the GIPR^{dn} transgenic & the WT pigs compared to the pigs on the CON diet. Spermidine is associated with cellular autophagy and an antioxidative effect [1]. The decrease of this compound may hint at its higher expenditure due to endoplasmatic reticulum stress during the LCMP diet. The decreased amino acids (AA) belong to the glucogenic AA, indicating an increased use of protein for gluconeogenesis in the absence of dietary carbs. Decreased plasma Gln levels have been associated with insulin resistance in humans [2]. Another cluster of metabolites was sign. higher in both LCMP groups than the CON groups. Among these were the α-aminoadipic acid (precursor of lysine), the aromatic AA, Gly, Orn, Lys, Met & the acylcarnitines C12, C18, C18:1, C2 and C16. Acylcarnitines are involved in mitochondrial energy metabolism, β-oxidation & the production of ketone bodies. The increase of these metabolites may be associated with fat as main energy source in the LCMP diet. The aromatic AA have been positively associated with the risk for diabetes [3], complementing the previously described reduced glucose tolerance at w4 on LCMP. There was a disruption of the PC pattern, some of which increased while others decreased at w4 in the LCMP group. The lysoPC/PC ratio was decreased at w4 in the LCMP pigs, which may be linked to a lower activity of inflammatory pathways. Most of the metabolomic alterations at w4 on LCMP diet were not present anymore at w8, indicating that the diet-induced changes were reversible to a high extent.

Conclusions: Feeding a LCMP diet to GIPR^{dn} transgenic & WT pigs clearly altered plasma metabolites. The AA patterns indicate a switch to endogenic gluconeogenesis during the low-carb diet. The diet effect seemed to be higher than the genotype effect, indicating the importance of nutritional approaches in diabetes models. We are grateful for the DFG for funding this project.

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Workshop

Potential and pitfalls of microbiome research – What to consider?

Potenzial und Stolpersteine der Mikrobiomforschung – Was zu beachten ist?

Zwirzitz B. – Vienna

Microbiome research has emerged as a pivotal field in understanding and optimizing animal nutrition, offering the potential to enhance feed efficiency, improve animal health, and reduce environmental impacts (Peixoto et al. 2021). The gastrointestinal microbiome plays a critical role in nutrient digestion, metabolism, and immune modulation, making it a prime target for interventions such as probiotics, prebiotics, and tailored feed additives (Kavanova et al. 2025; Shin et al. 2019). However, realizing the full potential of microbiome-based solutions requires addressing several critical challenges.

One of the key challenges in microbiome research is the temporal and spatial instability of microbial communities. The composition of the microbiome can vary significantly over time and across different sections of the gastrointestinal tract, complicating the interpretation of results and the establishment of consistent patterns (Liu et al. 2019; Marsh et al. 2024; Tropini et al. 2017). This instability is further influenced by a range of host-related and environmental factors, including antibiotic use, age, sex, diet, geography, host genetics, and housing conditions (Furman et al. 2020; Zwirzitz et al. 2023). These factors introduce variability that can obscure causative relationships and make it difficult to generalize findings across populations or farming systems. The lack of standardized methodologies exacerbates these challenges. Sampling protocols, storage conditions, and sequencing methodologies can all significantly affect the results of microbiome analyses (Koorakula et al. 2022). For instance, improper sample storage or delayed processing can alter the microbial composition, leading to biased or inaccurate data (Wegl et al. 2021). Similarly, the choice of primers and sequencing platforms can influence the resolution and coverage of microbial taxa, impacting the comparability of results between studies (Silverman et al. 2021). The inclusion of appropriate controls and replicates is also essential to account for inherent variability and to ensure the reliability of conclusions, yet these elements are often inconsistently implemented across studies (Salter et al. 2014). Another layer of complexity arises from host-microbiome interactions. The microbiome's functionality is not only shaped by its composition but also by its interplay with the host's immune system, physiology, and genetics (Ezequias et al. 2024; Steele et al. 2011). This underscores the importance of integrating microbiome research with host genetics, animal nutrition, and environmental science to develop comprehensive models that reflect real-world conditions.

To overcome these pitfalls, microbiome research must adopt rigorous and standardized approaches. Researchers should prioritize the harmonization of sampling, storage, and analysis protocols to improve reproducibility and comparability across studies. Longitudinal studies that incorporate diverse factors, such as age, diet, and housing conditions, are necessary to capture the dynamic nature of the microbiome and its interactions with the host. Moreover, integrating advanced analytical tools with robust experimental designs that include appropriate controls and replicates will enable more precise characterization of microbial functions and their contributions to host traits.

In conclusion, while microbiome research holds tremendous potential to transform farm animal feeding practices, it also faces significant scientific, methodological, and practical challenges. Addressing these challenges requires a concerted effort to develop standardized protocols, interdisciplinary research frameworks, and innovative tools. By doing so, microbiome research can unlock new avenues for improving animal productivity, health, and sustainability in modern agriculture.

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Standardization in pig microbiome research to improve reproducibility and comparability

Standardisierung der Mikrobiomforschung bei Schweinen zur Verbesserung der Reproduzier- und Vergleichbarkeit

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Microbiome research has transformed our understanding of health, disease, and productivity across several fields, including animal science. Despite its potential, microbiome studies face several challenges in reproducibility and comparability due to a lack of standardized methodologies and data reporting practices. These issues limit the integration of findings across studies, particularly in livestock microbiome research.

Standardization in livestock microbiome research includes various aspects of experimental design and data processing, including sample collection methods, storage conditions, DNA extraction protocols, sequencing strategies, data generation, processing, and sharing to facilitate reproducibility and interoperability across studies. Each step in this workflow introduces potential biases that can affect microbial community profiles and hide meaningful comparisons across studies (1). In addition to harmonizing experimental methodologies, standardization is essential in bioinformatics workflows. Differences in taxonomic annotation databases (e.g., SILVA, Greengenes) and functional annotation tools (e.g., KEGG, MetaCyc) introduce variability that complicates cross-study comparisons. To address this, metadata files must provide detailed documentation of computational tools, reference databases, and analysis parameters, ensuring that datasets are not only reproducible but also interoperable within the framework of FAIR principles (2).

A systematic review of 2,886 pig microbiome studies revealed substantial methodological variability (see abstract 81). Only 460 studies met the criteria of accessible metadata and publicly available sequencing data. A meta-analysis demonstrated that the choice of DNA extraction kit significantly affected microbial composition and diversity indices, even when targeting identical 16S rRNA gene regions and bioinformatics pipelines. To start addressing these issues, a metadata template was developed to standardize reporting, incorporating biological and experimental variables to improve cross-study comparability.

In conclusion, advancing animal microbiome research depends on establishing standardized protocols integrating species-specific requirements and metadata practices. These efforts will improve the reliability and interpretability of findings, facilitate study comparisons, and contribute to advancements in animal health, productivity, and sustainable agricultural practices.

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Microbiome between basic research and clinic - the field of companion animal medicine

Mikrobiom zwischen Grundlagenforschung und Klinik - Erkenntnisse aus dem Bereich der Kleintiermedizin

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The intestinal microbiome is a complex and dynamic ecosystem that plays a crucial role in the health and well-being of animals. It consists of a wide variety of microorganisms, including bacteria, viruses, fungi, and archaea, which interact closely with the host. This microbial community is often referred to as an "independent organ" due to the diverse and essential functions it performs, such as supporting digestion, modulating the immune system, and maintaining the barrier function of the intestinal mucosa. The development of the microbiome begins early in an animal's life and is a continuous process. Colonization of the gut starts in the first days of life, influenced by factors such as the mode of delivery (natural or cesarean) and is tightly dependent on the age of the animal. In young animals, bacterial groups such as Firmicutes, Clostridium perfringens, and Clostridium difficile dominate. Over time, the microbiome diversifies and stabilizes, which is essential for the animal's long-term health and immune system development. Diet was identified early on as a key factor influencing the intestinal microbiome in dogs (Torrey 1919). Dietary factors continue to be the focus of attention, particularly for the treatment of gastrointestinal diseases, which may be associated with an imbalance of the intestinal microbiome.

Modern analytical methods, particularly 16S-rRNA sequencing and metagenomic approaches, enable detailed investigation of the microbiome's composition and function. These technologies have revolutionized our understanding of microbial communities, allowing the identification of specific microbial changes associated with diseases or environmental factors. One frequently used practice parameter is the so-called “dysbiosis index”, which is often used to assesses microbial balance in the gastrointestinal tract of dogs and cats and allows for identifying imbalances often linked to chronic intestinal diseases (AlShawaqfeh et al. 2017).

A healthy, eubiotic microbiome is characterized by high diversity, stability, and the presence of beneficial microorganisms such as Lactobacillus and Bifidobacterium spp. These "good" bacteria promote host health by displacing pathogenic microbes, producing short-chain fatty acids (SCFAs) like butyrate, and positively modulating the immune system (Suchodolski 2022). Conversely, dysbiosis—an imbalance in microbial communities—can significantly impair intestinal health. Typical consequences of dysbiosis include disrupted intestinal barrier function, inflammation, and dysregulation of immune responses. Dysbiosis is often associated with conditions such as colitis, chronic inflammatory bowel diseases, or metabolic disorders.

Nutrition plays a key role in shaping and modulating the microbiome. Macronutrients such as proteins, carbohydrates, and fiber significantly influence the composition of the microbiota in dogs and cats. Fermentable carbohydrates and soluble fibers are often used to promote the production of SCFAs like butyrate, which have anti-inflammatory properties and strengthen the intestinal barrier. Conversely, an excessive proportion of low digestible proteins can encourage the growth of pathogenic microorganisms and negatively impact gut health (Zentek et al. 2003). A method of feeding that has become widespread in recent years, especially for dogs, is raw feeding (BARF). This has a lasting effect on the intestinal microbiome. On the one hand, there are shifts in the sense of intestinal dysbiosis, and on the other hand, pathogens, especially Salmonellae, can be transmitted through unheated feed. (Joffe and Schlesinger 2002, van Bree et al. 2018, Koch et al. 2020). In addition to a balanced diet, prebiotics, probiotics, and synbiotics offer promising approaches for targeted modulation of the microbiome. Probiotics such as Lactobacillus spp. and Bifidobacterium spp. are often used to strengthen the barrier function, improve microbial balance, and positively influence the immune system. However, recent evidence shows limited efficacy in gastrointestinal patients (Jensen and Bjornvad 2019, Scahill et al. 2024). Prebiotics, such as inulin or fructooligosaccharides, serve as selective nutrients for beneficial bacteria and promote their growth (Paßlack et al. 2021). Synbiotics combine the benefits of both prebiotics and probiotics, providing even more targeted support for gut health (Pilla et al. 2019). These approaches have been introduced in veterinary medicine for managing gastrointestinal conditions such as colitis or antibiotic-associated diarrhea. From the limited data available, it is clear that there is a need for further research under clinical conditions. The efficiency of the corresponding measures is generally not sufficient as a therapy.

In summary, the intestinal microbiome is considered to play a central role in animal health. It not only influences digestion and the immune system but also affects overall well-being and resilience to disease. Dysbiosis, or microbial imbalance, can probably lead to a wide range of health problems. On the other hand, targeted nutritional strategies provide effective ways to promote gut health and restore microbiome balance. Ongoing research in this field opens new perspectives for the prevention and treatment of microbiome-associated diseases and highlights the importance of personalized and scientifically-based nutrition in veterinary medicine.

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The microbiome of livestock – case study: dairy cows

Das Mikrobiom von Nutztieren – Fallstudie: Milchkühe

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The microbial communities (microbiomes) in the animal's digestive tract form a unique symbiosis with the host animal. They act as a central unit for maintaining the metabolic performance and the immune and nutritional status of the animal. The microbial diversity and functionality are important for the efficient utilisation of available feed resources and reduced formation of undesirable metabolic products. At the same time, priority should be given to animal health and animal welfare, which are the focus of current research activities alongside low-emission and resource-efficient animal production. Colonisation of the digestive tract begins at birth and is subject to dynamic development over the course of an animal's life. The composition of the microbiome varies from individual to individual and is strongly influenced by the animal's diet, environment and genetics. A possible change in the microbiome has an impact on the interaction of microorganisms with each other, as well as between microorganisms and the host animal, and thus on animal health.

Ruminants have a special symbiosis with their microbiome due to their diet and forestomach system. They are heavily dependent on the activity of microorganisms and the re-synthesis of microbial biomass. The rumen plays a key role in this. Here, ingested feed is converted by microbial activity and broken down into microbial metabolites. The conditions in the rumen develop dynamically during the first month of life (Amin & Seifert 2021) and are ideal for anaerobic microorganisms providing them with a continuous supply and removal of food and metabolic products.

The possible links between health, performance and the composition of the microbiome were identified in dairy cows in the collaborative project MitoCow (DFG funded no. 202989534). Bioinformatic analyses showed significant correlations between certain bacteria and the milk yield and health status of the animals by grouping them into microbial clusters (Tröscher-Mußotter et al. 2021). Relatives of bifidobacteria seems to play a key role during the entire live span of the animals. This study provides a first in-depth indication of the use of the microbiome as a possible selection marker in animal breeding. However, a better understanding of the structure and function of the microbiome and its internal and external influencing factors is essential in order to address these issues in the future.

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Equations for estimating organic matter digestibility of compound feeds for ruminants

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1. Introduction

The energy value of feedstuffs must be known to enable farm animals to be supplied with energy according to their requirement. In the case of ruminants, the energy is evaluated using metabolisable energy (ME). The ME of feeds is not exclusively but primarily influenced by energy digestibility. Energy digestibility is very closely related to organic matter digestibility (OMD) and determining OMD is the initial step in the calculation of ME in the *three-step procedure* introduced by GfE (2023). Two further calculation steps consider the energy lost via urine and methane.

Given the central role of OMD in the energy evaluation of feeds on the one hand and the great expense of measuring digestibility in animal experiments on the other, estimation methods are required with which the OMD can be determined under laboratory conditions. Hence, equations are provided here to estimate the OMD of compound feeds using chemical analyses and *in vitro* data. The evaluation is based on data from *in vivo* digestibility studies of compound feeds formulated for supplementation in the feeding of dairy cows, beef cattle, calves, and sheep. According to the methodological guidelines of GfE (1991), such feeds are tested in a 'difference' trial, meaning they are assessed in a defined mixed ration with a forage component. This approach was also applied to the compound feeds in the present dataset. As *in vitro* criteria, the enzyme-soluble organic matter (ESOM) and 24-h gas production (GP) determined in the Hohenheim gas test were alternatively used. The underlying data originate from digestibility trials with wethers, all conducted at the same facility under standardised conditions.

Abbreviations: **ADFom** = acid detergent fibre expressed exclusive of residual ash; **aNDFom** = neutral detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash; **CL** = crude fat; **CP** = crude protein; **DM** = dry matter; **ESOM** = enzyme-soluble organic matter; **GP** = gas production; **GE** = gross energy; **ME** = metabolizable energy; **n** = number of observations **OM** = organic matter; **OMD** = organic matter digestibility; **OR** = organic residue; **R^2** = coefficient of determination; **RMSE** = Root Mean Square Error; **RSD** = residual standard deviation; **SD** = standard deviation; **ST** = starch; **SU** = sugar

2. Data and procedure

For the analysis, data from 299 digestibility trials conducted between 2014 and 2022 at the Landwirtschaftskammer Nordrhein-Westfalen in the Versuchs- und Bildungszentrum Landwirtschaft Haus Riswick were used. The selection of data adhered to the criteria that all predictor variables relevant to OMD were analysed in all feeds, and that the neutral detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash (aNDFom) was consistently analysed according to the VDLUFA method. The dataset included 237 compound feeds for dairy cows, 43 for beef cattle and calves, and 19 for sheep. The chemical composition of these compound feeds and the *in vitro* data were determined using standard procedures or calculated based on those procedures and the results are summarised in Table 1.

Table 1: Analysed constituents, *in vitro* data and *in vivo* organic matter digestibility of the compound feeds in the data set (mean, standard deviation SD, coefficient of variation SD%, minimum and maximum; n = 299 feeds)

		Mean	SD	SD%	Min	Max
Ash		73	12.3	16.9	35	122
Crude protein		224	37.4	16.7	94	418
Crude fat		44	8.7	20.0	24	80
Starch		271	76.2	28.1	31	511
Sugar	g/kg DM	75	20.7	27.5	19	140
Organic residue		313	56.6	18.1	187	474
aNDFom		270	53.2	19.7	145	418
ADFom		147	32.5	22.1	70	246
ESOM		801	46.9	5.9	651	893
Gas production	mL/200 mg DM	58	4.2	7.2	42	69
OMD	%	83.6	3.4	4.0	68.5	91.3

With coefficients of variation between 20 and 22% for the fibre fractions, 17% for crude protein, and 20% for crude fat, the data variation for the derivation of regression equations was high as intended. The variation was smaller for ESOM (6%), GP (7%) and OMD (4%). The estimation equations were derived based on the OMD measured in the digestibility trials, the analysed constituents (CP, CL, ADFom, aNDFom, starch and sugar in g/kg organic matter; OM) and the *in vitro* variables GP (mL/200 mg OM) and ESOM (g/kg OM). By relating the variables to the OM, it was avoided that the ash concentration in the regression equation may have a mathematical influence on the OMD without a direct causal link.

For the mathematical derivation of the estimation equations, the individual data from the digestibility trials were not used directly; instead, variable mean values classified by OMD were employed. The reason for this approach was the absence of a normal distribution of the data across the range of OMD values (Figure 1). The lower half of the data sorted by OMD covered the range of 68.5 – 84.2%, whereas the upper half spanned a narrower range of 84.2 – 91.3%. Consequently, there was a higher data density in the upper digestibility range, which influenced the regression coefficients when linear relationships between OMD and the individual predictor variables were calculated. This effect was avoided by classifying the dataset, ensuring a more balanced representation of data points across the range of OMD values.

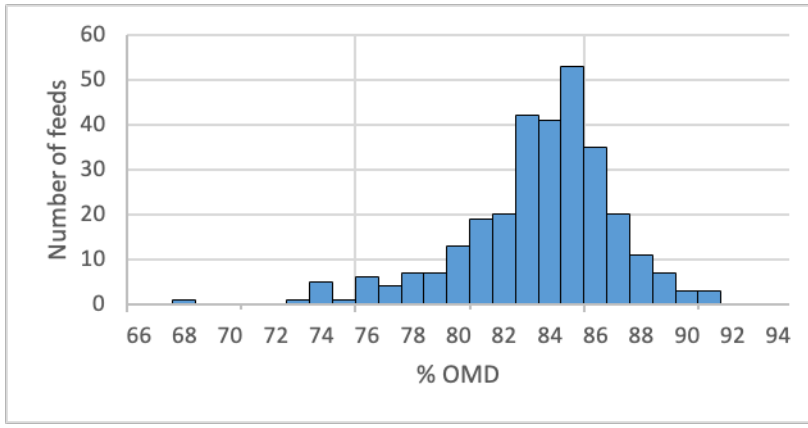

Figure 1: Frequency distribution of datasets classified by organic matter digestibility (OMD)

For the mathematical derivation of the estimation equations, the 299 datasets were aggregated into classes based on rolling averages of OMD, following the approach used in previous studies (GfE 2024). Corresponding variables were averaged in the same manner. The lowest class comprised datasets with OMD ≤ 76%. Classes were constructed with a span of five percentage points of OMD, formed at intervals of one percentage point using rolling averages (Table 2). The highest class included datasets with an OMD ≥ 89%.

Table 2: Mean values of the data sets classified according to OMD

OMD class	n	OMD	CP	CL	ST	SU	aNDFom	ADFom	ESOM	GP
										mL/200
%		%				g/kg OM				mg OM
≤ 76	9	73.7	248	49	193	80	382	212	781	52.9
72 – 77	14	75.2	254	48	189	82	381	211	794	54.5
73 – 78	20	76.2	252	47	191	86	377	211	802	55.8
74 – 79	27	76.8	251	50	197	83	371	206	806	56.1
75 – 80	32	78.2	249	51	214	81	355	197	811	57.4
76 – 81	45	79.0	257	52	214	81	347	193	820	57.6
77 – 82	60	80.1	254	51	229	81	339	185	824	58.7
78 – 83	83	81.2	248	51	252	79	326	176	836	59.9
79 – 84	118	82.2	245	49	270	78	314	169	844	61.1
80 – 85	159	83.1	242	48	283	79	304	163	855	62.1
81 – 86	189	83.9	239	47	298	80	293	157	864	62.9
82 – 87	197	84.5	238	46	306	82	283	153	873	63.4
83 – 88	177	85.0	238	45	314	82	275	150	879	63.9
84 – 89	147	85.7	238	45	321	84	266	145	885	64.5
85 – 90	100	86.5	235	45	335	86	252	139	896	65.0
86 – 91	61	87.5	240	45	346	86	235	130	906	65.5
87 – 92	34	88.5	242	45	354	84	225	123	913	66.3
88 – 93	20	89.2	248	47	365	79	213	111	912	66.4
≥ 89	10	90.0	254	49	385	73	192	102	937	66.4

For the analysis of statistical relationships with OMD, all available predictor variables were initially used: CP, CL, ST, SU, aNDFom, ADFom, and ELOS (each in g/kg OM), as well as GP (in mL/200 mg OM). However, it is emphasised that only one of the two *in vitro* criteria was included in the model at a time. Applying the stepwise approach, the calculations were performed using SAS® (Version 9.4) with the PROC REG procedure. A significance level of $p \leq 0.15$ was used as the threshold for the inclusion or retention of a predictor variable. The coefficient of determination (R^2) and the residual standard deviation (RSD) were calculated based on the application of the estimation parameters determined with the rolling class averages to the individual datasets (functional values). The resulting estimation equations were compared using the calculated R^2 and RSD values.

3. Equations and their scopes

Estimation equations that did not contain an *in vitro* criterion as a predictor variable were significantly worse in the goodness of fit than those with an *in vitro* criterion and are not reported here. The two equations with the highest prediction accuracy were the following.

[1] OMD (%) = 26.9
+ 0.0342 • CP (g/kg OM)
+ 0.0706 • CL (g/kg OM)
− 0.0254 • aNDFom (g/kg OM)
+ 0.842 • GP (mL/200 mg OM)

R^2 = 0.72; RMSE = 2.1; RSD in % of the mean = 2.5; n = 299

[2] OMD (%) = −8.3
+ 0.106 • ESOM (g/kg OM)

R^2 = 0.49; RMSE = 3.6; RSD in % of the mean = 4.3; n = 299

Based on the range of values in the data pool used to derive the equations (Table 1), the following ranges of values apply when using the equations:

ESOM:	650 – 890	g/kg DM
GP:	40 – 70	mL/200 mg DM
ADFom:	70 – 245	g/kg DM
aNDFom:	145 – 420	g/kg DM
CL:	up to 80	g/kg DM
CP:	up to 420	g/kg DM
ST:	30 – 510	g/kg DM
SU:	20 – 140	g/kg DM
Ash:	up to 120	g/kg DM

If the analysed values of a feed sample fall outside of these ranges, the accuracy of the OMD estimation decreases. This fact must be pointed out when reporting the results.

Using urea as an additive in compound feed results in a lower GP in the Hohenheim gas test that is not reliably quantifiable. Hence, a reduced accuracy of the OMD estimation using Equation [1] is expected for compound feed with higher levels of urea inclusion.

When using ESOM as a predicting variable, the inclusion of other analysed fractions did not lead to an increase in the quality of the estimation. The determination of aNDFom and ADFom is, therefore, not necessary for the energetic feed evaluation if ESOM is used. This does not affect the need to determine these fractions for other feed evaluation purposes.

The dataset consisted predominantly of compound feeds for dairy cows. A differentiated evaluation for the groups of compound feeds showed that the equations could generally be applied to compound feeds for beef cattle, calves, and sheep as well (Table 3). When using Equation [2], the OMD values – similar to the results for the overall dataset – tend to be underestimated on average for dairy cow compound feeds and, more notably, beef cattle and calf compound feeds. Conversely, sheep compound feeds are overestimated by approximately one percentage point of OMD when using Equation [2].

When applying Equation [1], a noticeable bias is observed for sheep compound feeds, where OMD is underestimated by about one percentage point on average.

Table 3: Analysed constituents, *in vitro* data, organic matter digestibility, and prediction accuracy for compound feeds differentiated in dairy cow, beef cattle, calf, and sheep feeds

		Total	Dairy	Beef/Calf	Sheep
Number of feeds		299	237	43	19
$OMD_{in\ vivo}$	%	83.6	84.2	81.9	80.0
Ash	g/kg DM	73	69	88	86
CP	g/kg OM	242	237	279	218
CL	g/kg OM	47	47	49	42
ST	g/kg OM	292	306	245	218
SU	g/kg OM	81	80	83	94
aNDFom	g/kg OM	292	284	302	369
ADFom	g/kg OM	159	155	160	202
ESOM	g/kg OM	863	870	839	843
GP	mL/200 mg OM	62.4	63.1	59.5	60.5
$Bias_{Eq.\ [1]}$	% OMD	0.0	0.0	0.4	−1.0
RMSE	% OMD	2.1	2.1	2.2	2.4
$Bias_{Eq.\ [2]}$	% OMD	−0.4	−0.3	−1.2	1.1
RMSE	% OMD	3.6	3.4	4.7	3.5

Recommendation for estimating OMD

Due to the significant differences in the R^2 and RSD values between the two equations, as well as the systematic bias observed with Equation [2], it is generally recommended to use Equation [1]. If Equation [2] must be used, due to the availability of only ESOM as the *in vitro* criterion, a greater estimation error in determining OMD should be anticipated.

Regarding compound feeds for sheep, both equations resulted in a mean under- or overestimation of OMD by approximately one percentage point. Additional digestibility trials, particularly but not exclusively for this group of compound feeds, are encouraged to improve the predictive accuracy of the equations. Expanding the dataset with more comprehensive trials will help refine the equations and reduce bias for specific feed types, enhancing their reliability for practical application. Future studies should also consider the effects of added urea in compound feeds on GP and OMD estimation.

The equations were derived using data from compound feeds. Their application to compound feed ingredients frequently leads to marked errors. The equations are, therefore, not suitable for estimating the OMD of feed ingredients.

4. Calculation of ME

The *three-stage procedure* for calculating the ME was introduced with the revision of the supply recommendations for dairy cows (GfE 2023). For several feeds, this procedure leads to different ME values than the previously used procedure, in which the ME was calculated from digestible crude nutrients (GfE 2001). With the introduction of the *three-stage procedure*, the previously recommended equations for estimating the ME in compound feeds for cattle are no longer valid (GfE 2009). In the future, the equations presented here for estimating the OMD and the equation from GfE (2023) for the further calculation of the ME will apply:

$$\text{ME (MJ/kg DM)} = [(\text{GE (MJ/kg OM)} \bullet (\text{OMD (\%)} - 3.3) \div 100 - 0.0037 \bullet \text{CP (g/kg OM)} - (0.7 + 0.014\ \text{OMD (\%)}))] \bullet (1 - \text{ash (g/kg DM)} \div 1000)$$

The gross energy is determined using calorimetry, considering the analysed ash concentration (GE, MJ/kg OM). Calorimetry is particularly useful for compound feeds with added urea. If a calorimetric determination is not possible, gross energy can be calculated using the following equations:

Compound feeds without added urea

GE (kJ/kg OM) = (23.6 CP + 39.8 CL + 17.3 starch + 16.0 sugar + 18.9 OR) ÷ (1 − ash ÷ 1000)
where OR (organic residue) = OM − CP − CL − starch – sugar (g/kg DM).

Compound feeds with added urea

GE (kJ/kg OM) = [23.6 (total N – urea-N) • 6.25 + 10.5 urea + 39.8 CL + 17.3 starch + 16.0 sugar + 18.9 OR´] ÷ (1 − ash ÷ 1000)
where OR´ (organic residue) = OM − (total N – urea-N) • 6.25 – urea − CL − starch – sugar (g/kg DM).

5. References

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Gleichungen zur Schätzung der Verdaulichkeit der Organischen Masse von Mischfuttermitteln für Wiederkäuer

Empfohlene Zitierweise:
GfE [Gesellschaft für Ernährungsphysiologie] 2025. Gleichungen zur Schätzung der Verdaulichkeit der Organischen Masse von Mischfuttermitteln für Wiederkäuer. Proc. Soc. Nutr. Physiol. 34:145-151.

1. Einleitung

Für eine bedarfsgerechte Energieversorgung landwirtschaftlicher Nutztiere muss der energetische Futterwert bekannt sein. Bei Wiederkäuern ist die Umsetzbare Energie (ME) der in Deutschland und vielen anderen Ländern gültige Maßstab zur Bewertung des Energiebedarfs und des Energiegehalts von Futtermitteln. Die ME eines Futtermittels wird zwar nicht ausschließlich, jedoch zuvorderst durch die Verdaulichkeit der Energie beeinflusst. Die Verdaulichkeit der Energie steht in einer sehr engen Beziehung zur Verdaulichkeit der Organischen Masse (Organic Matter Digestibility, OMD), so dass die Bestimmung der OMD der initiale Schritt bei der Berechnung der ME im *dreistufigen Verfahren* ist (GfE 2023). Die Energieabgabe über den Harn und als Methan wird in zwei weiteren Berechnungsschritten berücksichtigt.

Angesichts der zentralen Bedeutung der OMD bei der energetischen Futterbewertung einerseits und des hohen Aufwandes zur Messung der Verdaulichkeit mit Tieren andererseits werden Schätzverfahren benötigt, mit denen die OMD unter Laborbedingungen ermittelt werden kann. Im Folgenden werden Gleichungen vorgestellt, mit denen die OMD von Mischfuttermitteln – hergestellt aus Konzentratfuttermitteln – auf der Basis von chemischen Analysen und zwei voneinander unabhängigen *in-vitro*-Verfahren geschätzt wird. Die Auswertung basiert auf Daten aus *in-vivo*-Verdaulichkeitsuntersuchungen von Mischfuttermitteln, die zur Ergänzung in Rationen für Milchkühe, Mastrinder, Kälber und Schafe genutzt werden. Gemäß der Methodenvorschrift (GfE 1991) werden Futtermittel, die nicht als Alleinfutter eingesetzt werden, im „Differenzversuch", d. h. in einer definierten Mischration zusammen mit einem Grobfutter geprüft, was auch für die Mischfuttermittel des hier verwendeten Datensatzes galt. Als *in-vitro*-Kriterien wurden alternativ die enzymlösliche Organische Substanz (ELOS) und die im Hohenheimer Futterwerttest bestimmte Gasbildung nach 24 Stunden (GB) verwendet. Die zugrundeliegenden Daten stammen aus Verdaulichkeitsversuchen mit Hammeln, die alle in derselben Einrichtung unter weitgehend standardisierten Bedingungen durchgeführt wurden.

Verwendete Abkürzungen: **ADFom** = Säure-Detergenzien-Faser nach Veraschung; **aNDFom** = Neutral-Detergenzien-Faser nach Amylase-Behandlung und Veraschung; **CA** = Rohasche; **CL** = Rohfett; **CP** = Rohprotein; **ELOS** = Enzymlösliche Organische Substanz; **GB** = Gasbildung; **GE** = Bruttoenergie; **ME** = Umsetzbare Energie; **OM** = Organische Masse; **OMD** = Verdaulichkeit der Organischen Masse; **OR** = Organischer Rest; **R^2** = Bestimmtheitsmaß; **RMSE** = Root Mean Square Error; **RSD** = Residuale Standardabweichung; **SD** = Standardabweichung; **ST** = Stärke; **TM** = Trockenmasse; **ZU** = Zucker

2. Daten und Vorgehensweise

Für die Auswertung wurden die Daten aus 299 Verdaulichkeitsversuchen verwendet, die im Zeitraum von 2014 bis 2022 von der Landwirtschaftskammer Nordrhein-Westfalen im Versuchs- und Bildungszentrum Landwirtschaft Haus Riswick durchgeführt wurden. Die Auswahl der Daten beruhte auf den Vorgaben, dass alle für die OMD in Frage kommenden Vorhersagegrößen vollständig analysiert wurden sowie die Analytik der Neutral-Detergenzien-Faser gesichert nach Amylase-Behandlung und Veraschung des Analysenrückstandes (aNDFom) gemäß der VDLUFA-Methode erfolgte. Der Datensatz beinhaltet 237 Milchleistungs- (MLF), 43 Rindermast- und Kälber- (RMK) sowie 19 Schaffuttermittel (SCH). Die Inhaltsstoffe sowie die *in-vitro*-Daten zu ELOS und GB wurden unter Verwendung der jeweils gültigen Standardverfahren ermittelt bzw. auf deren Grundlage berechnet. In der Tabelle 1 sind die Daten zur Charakterisierung der Mischfuttermittel zusammengefasst.

Tabelle 1: Inhaltsstoffe, *in-vitro*-Daten und *in-vitro*-Verdaulichkeit der Organischen Masse im gesamten Datensatz (Mittelwert, Standardabweichung SD, Variationskoeffizient SD%, Minimum und Maximum, n =299 Mischfuttermittel)

		Mittelwert	SD	SD%	Min	Max
Rohasche		73	12,3	16,9	35	122
Rohprotein		224	37,4	16,7	94	418
Rohfett		44	8,7	20,0	24	80
Stärke		271	76,2	28,1	31	511
Zucker	g/kg DM	75	20,7	27,5	19	140
Organischer Rest		313	56,6	18,1	187	474
aNDFom		270	53,2	19,7	145	418
ADFom		147	32,5	22,1	70	246
ELOS		801	46,9	5,9	651	893
Gasbildung	mL/200 mg DM	58	4,2	7,2	42	69
OMD	%	83,6	3,4	4,0	68,5	91,3

Das Datenmaterial wies mit Variationskoeffizienten zwischen 20 und 22 % für die Faserfraktionen, 17 % für Rohprotein und 20 % für Rohfett eine angestrebt hohe Variation zur Ableitung von Regressionsgleichungen auf. Mit etwa 6 % für ELOS, 7 % für GB sowie 4 % für die OMD war die Variation bei diesen Variablen geringer. Die Ableitung der Schätzgleichungen erfolgte auf der Basis der in den Verdaulichkeitsversuchen gemessenen OMD, der analysierten Inhaltsstoffe (CP, CL, ADFom, aNDFom, ST und ZU in g/kg Organische Masse; OM) und der *in-vitro*-Größen GB (mL/200 mg OM) und ELOS (g/kg OM). Durch den Bezug der Inhaltsstoffe und der *in-vitro*-Daten auf die OM wurde vermieden, dass die Rohasche-Konzentration in der Regressionsgleichung rechnerisch einen Einfluss auf die OMD bekommt, der nicht sachlogisch wäre.

Für die mathematische Ableitung der Schätzgleichungen wurden nicht die Daten eines jeden Verdaulichkeitsversuches einzeln verwendet, sondern jeweilige Variablen-Mittelwerte der nach OMD klassifizierten Datensätze. Der Grund für diese Herangehensweise war die fehlende Normalverteilung der Daten über den gesamten Bereich der ermittelten OMD (Abbildung 1). Diese Verteilung beinhaltete, dass die untere Hälfte der nach OMD sortierten Daten einen Bereich von 68,5 – 84,2 % abdeckte, während die obere Hälfte einen deutlich kleineren Bereich von 84,2 – 91,3 % abdeckte. Daraus resultierte eine höhere Datenhäufigkeit im oberen Bereich der Verdaulichkeit, die sich bei der linearen Beschreibung grundlegender Zusammenhänge zwischen der OMD und den einzelnen Variablen gerichtet auf die Regressionskoeffizienten auswirkte. Dieser Effekt konnte mit der Klassifizierung des Datensatzes umgangen werden.

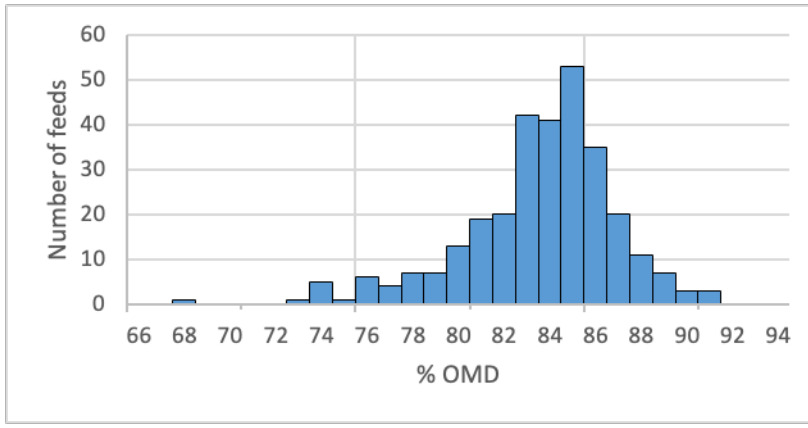

Abbildung 1: Häufigkeitsverteilung der Datensätze nach Klassen der Verdaulichkeit der Organischen Masse (OMD)

Für die mathematische Ableitung der Schätzgleichungen wurden die 299 Datensätze in Klassen anhand gleitender Mittelwerte der OMD aggregiert, wie dies bereits in früheren Arbeiten erfolgte (GfE 2024). Für die dazugehörigen Variablen wurden in gleicher Weise Mittelwerte gebildet. Die kleinste Klasse bildeten die Datensätze mit OMD ≤ 76 %. Die Klassen wurden mit einer Spannweite der OMD von 5 %-Einheiten im Abstand von einer %-Einheit OMD mit gleitenden Mittelwerten gebildet (Tabelle 2). Die letzte Klasse enthielt die Datensätze mit einer OMD ≥ 89 %.

Tabelle 2: Gleitende Mittelwerte der Datensätze nach Klassen der OMD

Klasse OMD %	n	OMD %	CP	CL	ST	ZU	aNDFom	ADFom	ELOS	GB mL/200 mg OM
						g/kg OM				
≤ 76	9	73,7	248	49	193	80	382	212	781	52,9
72 – 77	14	75,2	254	48	189	82	381	211	794	54,5
73 – 78	20	76,2	252	47	191	86	377	211	802	55,8
74 – 79	27	76,8	251	50	197	83	371	206	806	56,1
75 – 80	32	78,2	249	51	214	81	355	197	811	57,4
76 – 81	45	79,0	257	52	214	81	347	193	820	57,6
77 – 82	60	80,1	254	51	229	81	339	185	824	58,7
78 – 83	83	81,2	248	51	252	79	326	176	836	59,9
79 – 84	118	82,2	245	49	270	78	314	169	844	61,1
80 – 85	159	83,1	242	48	283	79	304	163	855	62,1
81 – 86	189	83,9	239	47	298	80	293	157	864	62,9
82 – 87	197	84,5	238	46	306	82	283	153	873	63,4
83 – 88	177	85,0	238	45	314	82	275	150	879	63.9
84 – 89	147	85,7	238	45	321	84	266	145	885	64,5
85 – 90	100	86,5	235	45	335	86	252	139	896	65,0
86 – 91	61	87,5	240	45	346	86	235	130	906	65,5
87 – 92	34	88,5	242	45	354	84	225	123	913	66,3
88 – 93	20	89,2	248	47	365	79	213	111	912	66,4
≥ 89	10	90,0	254	49	385	73	192	102	937	66,4

Für die Auswertung statistischer Zusammenhänge zur OMD wurden zunächst alle verfügbaren Vorhersagegrößen als Variablen genutzt: CP, CL, ST, ZU, aNDFom, ADFom, ELOS (jeweils in g/kg OM), GB (in mL/200 mg OM), allerdings war immer nur eines der beiden *in-vitro*-Kriterien zugelassen.

Die Berechnungen wurden mittels SAS® (Version 9.4) unter Nutzung der Prozedur PROC REG im stepwise-Verfahren vorgenommen. Dabei wurde eine Irrtumswahrscheinlichkeit von $p \leq 0{,}15$ zur Aufnahme oder Beibehaltung einer Vor-

hersagegröße angenommen. Das Bestimmtheitsmaß (R^2) und die Residuale Standardabweichung (RSD) wurden auf Basis der Anwendung der mit den gleitenden Klassenmittelwerten ermittelten Schätzparameter auf die individuellen Datensätze (Funktionswerte) berechnet. Die sich ergebenden Schätzgleichungen wurden jeweils anhand des so berechneten R^2 und der RSD miteinander verglichen.

3. Ergebnisse und Gültigkeitsbereich der Gleichungen

Schätzgleichungen, die kein *in-vitro*-Kriterium als Variable enthielten, waren in den Güteparametern deutlich schlechter als diejenigen mit einem *in-vitro*-Kriterium und werden nachfolgend nicht wiedergegeben. Die unter Beachtung der Werte für R^2 und RSD vorzüglichen Gleichungen waren die beiden folgenden:

[1] OMD (%) = 26,9
+ 0,0342 • CP (g/kg OM)
+ 0,0706 • CL (g/kg OM)
− 0,0254 • aNDFom (g/kg OM)
+ 0,842 • GB (mL/200 mg OM)

R^2 = 0,72; RMSE = 2,1; RSD in % of the mean = 2,5; n = 299

[2] OMD (%) = −8,3
+ 0,106 • ELOS (g/kg OM)

R^2 = 0,49; RMSE = 3,6; RSD in % of the mean = 4,3; n = 299

Auf Basis der Wertebereiche in dem für die Ableitung der Gleichungen genutzten Datensatz (Tabelle 1) wird folgender Geltungsbereich für die Schätzung der OMD mit den Gleichungen [1] und [2] angegeben:

ELOS:	650 – 890	g/kg DM
GB:	40 – 70	mL/200 mg DM
ADFom:	70 – 245	g/kg DM
aNDFom:	145 – 420	g/kg DM
CL:	bis 80	g/kg DM
CP:	bis 420	g/kg DM
ST:	30 – 510	g/kg DM
ZU:	20 – 140	g/kg DM
CA:	bis 120	g/kg DM

Falls die Analysenergebnisse einer Futterprobe außerhalb dieser Bereiche liegen, nimmt die Genauigkeit der Schätzung ab. Bei der Ausweisung der Ergebnisse ist auf diesen Sachverhalt hinzuweisen.
Der Einsatz von Futterharnstoff in Mischfuttermitteln bewirkt eine nicht sicher quantifizierbar geringere GB im Hohenheimer Futterwerttest. Bei Mischfuttermitteln mit höheren Anteilen an Futterharnstoff ist daher eine verminderte Genauigkeit der OMD-Schätzung mit der Gleichung [1] zu erwarten.
Bei Verwendung von ELOS führte eine Einbeziehung weiterer Variablen nicht zu einer Erhöhung der Schätzgüte. Die Bestimmung von aNDFom und ADFom ist somit für die energetische Bewertung von Mischfuttermitteln nicht erforderlich, wenn die ELOS bestimmt und die Gleichung [2] verwendet wird.

Die Notwendigkeit der Bestimmung dieser Fraktionen für andere Zwecke der Futterbewertung bleibt hiervon unberührt.
Der verwendete Datensatz bestand zum weit überwiegenden Teil aus MLF. Eine differenzierte Auswertung nach den Mischfuttermittelgruppen zeigte, dass die Gleichungen grundsätzlich auch für Rindermast-, Kälber- und Schafmischfutter angewendet werden können (Tabelle 3). Bei Nutzung von Gleichung [2] werden die OMD-Werte – in ähnlicher Weise wie für den gesamten Datensatz – für MLF und, deutlicher, für Rindermast- und Kälbermischfuttermittel im Mittel unterschätzt. Mischfuttermittel für Schafe hingegen werden durch Gleichung [2] um etwa eine %-Einheit OMD überschätzt. Bei Anwendung von Gleichung [1] zeigt sich bei den letztgenannten ebenfalls ein systematischer Fehler (Bias); die OMD wird hier im Mittel um eine %-Einheit unterschätzt.

Tabelle 3: Inhaltsstoffe, *in-vitro*-Daten, Verdaulichkeit der Organischen Masse (OMD) und Schätzgüte in dem nach Milchkühen, Mastrindern und Kälbern sowie Schafen differenzierten Datensatz

			Mischfuttermittel für		
		gesamt	Milchkühe	Mastrinder/Kälber	Schafe
Anzahl Futter		299	237	43	19
$OMD_{in\text{-}vivo}$	%	83,6	84,2	81,9	80,0
CA	g/kg DM	73	69	88	86
CP	g/kg OM	242	237	279	218
CL	g/kg OM	47	47	49	42
ST	g/kg OM	292	306	245	218
ZU	g/kg OM	81	80	83	94
aNDFom	g/kg OM	292	284	302	369
ADFom	g/kg OM	159	155	160	202
ELOS	g/kg OM	863	870	839	843
GB	mL/200 mg OM	62,4	63,1	59,5	60,5
$Bias_{Gleichung\ [1]}$	% OMD	0,0	0,0	0,4	−1,0
RMSE	% OMD	2,1	2,1	2,2	2,4
$Bias_{Gleichung\ [2]}$	% OMD	−0,4	−0,3	−1,2	1,1
RMSE	% OMD	3,6	3,4	4,7	3,5

Abschließende Empfehlung zur Schätzung der OMD

Aufgrund der deutlich unterschiedlichen Werte für das Bestimmtheitsmaß und die RSD zwischen den beiden Gleichungen und des systematischen Fehlers der Gleichung [2] wird grundsätzlich empfohlen, die Gleichung [1] zu verwenden. Falls die Gleichung [2] verwendet wird, weil als *in-vitro*-Kriterium nur ELOS zur Verfügung steht, ist ein größerer Schätzfehler bei der Bestimmung der OMD zu akzeptieren. Mischfuttermittel für Schafe werden mit beiden Gleichungen im Mittel um 1 %-Einheit OMD entweder unter- oder überschätzt (Tabelle 3). Es werden weitere Verdaulichkeitsversuche, insbesondere, aber nicht nur, für diese Gruppe von Mischfuttermitteln empfohlen, damit die Vorhersagegenauigkeit der Gleichungen erhöht werden kann. Zukünftige Untersuchungen sollten auch die Wirkungen der Verwendung von Futterharnstoff in Mischfuttermitteln auf die GB und die OMD-Schätzung beinhalten.

Die Gleichungen wurden mit Daten von Mischfuttermitteln abgeleitet. Die Anwendung auf Einzelfuttermittel führt in vielen Fälle zu erheblichen Fehleinschätzungen. Die Gleichungen sind daher für die Schätzung der OMD von Einzelfuttermitteln nicht geeignet.

4. Berechnung der ME

Mit der Überarbeitung der Versorgungsempfehlungen für Milchkühe wurde das *dreistufige Verfahren* zur Berechnung der ME eingeführt (GfE 2023). Dieses Verfahren führt bei einer Reihe von Futtermitteln zu anderen ME-Werten als das zuvor verwendete Vorgehen, bei dem die ME aus den verdaulichen Rohnährstoffen berechnet wurde (GfE 2001). Mit der Einführung des *dreistufigen Verfahrens* verliert daher die Gleichung an Gültigkeit, die zuvor für die Schätzung der ME in Mischfuttermitteln für Wiederkäuer empfohlen wurde (GfE 2009). Zukünftig gilt die hier vorgestellte Gleichung zur Schätzung der OMD. Für die weiterführende Berechnung der ME wird die Gleichung der GfE (2023) verwendet:

$$\text{ME (MJ/kg TM)} = [(\text{GE (MJ/kg OM)} \bullet (\text{OMD (\%)} - 3{,}3) \div 100 - 0{,}0037 \bullet \text{CP (g/kg OM)} - (0{,}7 + 0{,}014\ \text{OMD (\%)}))] \bullet (1 - \text{CA (g/kg TM)} \div 1000)$$

Die Bestimmung der Bruttoenergie erfolgt hierbei bevorzugt mittels Kalorimetrie unter Berück-sichtigung der CA-Analyse (GE, MJ/kg OM). Die Kalorimetrie ist insbesondere bei Mischfuttermitteln vorzüglich, die Futterharnstoff enthalten. Wenn eine kalorimetrische Bestimmung nicht möglich ist, kann eine Berechnung mit den folgenden Gleichungen erfolgen:

Mischfuttermittel ohne Futterharnstoff

$$\text{GE (kJ/kg OM)} = (23{,}6\ \text{CP} + 39{,}8\ \text{CL} + 17{,}3\ \text{Stärke} + 16{,}0\ \text{Zucker} + 18{,}9\ \text{OR}) \div (1 - \text{CA} \div 1000)$$

wobei OR (Organischer Rest) = OM − CP − CL − Stärke – Zucker (g/kg TM).

Mischfuttermittel mit Futterharnstoff

$$\text{GE (kJ/kg OM)} = [23{,}6\ (\text{Gesamt-N} - \text{Harnstoff-N}) \bullet 6.25 + 10{,}5\ \text{Harnstoff} + 39{,}8\ \text{CL} + 17{,}3\ \text{Stärke} + 16{,}0\ \text{Zucker} + 18{,}9\ \text{OR}'] \div (1 - \text{CA} \div 1000)$$

wobei OR´ (Organischer Rest) = OM − (Gesamt-N – Harnstoff-N) • 6.25 – Harnstoff − CL − Stärke – Zucker (g/kg TM).

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The three-step procedure for determining the metabolisable energy of feeds for horses

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Overview:

Applying the *three-stage procedure* to determine the content of metabolisable energy

The following summarises the stages and aspects relevant to the procedure. Detailed explanations of the approach and its implications are given in the subsequent sections.

The ***three-stage procedure*** for determining the metabolisable energy (ME) value of feed involves separately calculating the energy lost through faeces, urine and CH_4. This gives the procedure a clear structure and allows for further development and refinement of the calculation of energy losses. Energy digestibility (ED), energy lost through urine (UE, urinary energy) and through methane (CH_4-E) release are calculated as follows:

$$ED\ (\%) = OMD\ (\%) - 3.4$$

$$UE\ (MJ/kg\ DM) = 0.33 + 0.0043\ CP\ (g/kg\ DM)$$

$$CH_4\text{-}E = 0.83\ kJ/g\ OR$$

where DM is dry matter, CP is crude protein, and OR is organic residue (= OM – CP – CL – ST – SU). Accordingly, the ME content of a feed or ration is calculated using the following formula:

$$ME\ (MJ) = GE\ (MJ) \cdot [OMD\ (\%) - 3.4] \div 100 - [0.33 + 0.0043\ CP\ (g)] - 0.00083\ OR\ (g)$$

(all concentrations refer to 1 kg of DM)

It is recommended to use calorimetry to determine the heat of combustion (GE, gross energy). However, it is also possible to reliably estimate the GE of many feeds from their nutrient composition. This *three-stage procedure* replaces the procedure described by the GfE (2014) for estimating the ME of feeds and rations based on their concentrations of crude protein, crude lipid, crude fibre, and N-free extract.

Abbreviations: **ADF** Acid Detergent Fibre; **AfBN** Committee for Feeding Requirement Standards; **BW** Body Weight; **CA** Crude Ash; **CF** Crude Fibre; **CP** Crude Protein; **CH_4-E** methane energy; **CL** Crude Lipid; **CONC** Concentrate Feed; **DE** Digestible Energy; **DM** Dry Matter; **DMD** Dry Matter Digestibility; **DOM** Digestible Organic Matter; **ED** Energy Digestibility; **FIL** Feed Intake Level (FIL1 = 50 g DM/kg$^{0.75}$ BW; FILx = x · FIL1); **GE** Gross Energy; **GfE** German Society for Nutritional Physiology; **H_2-E** energy lost through hydrogen release; **ME** Metabolisable Energy; **NDF** Neutral Detergent Fibre; **NfE** N-free Extracts; **OM** Organic Matter; **OMD** Organic Matter Digestibility; **OMDh** OMD horse; **OMDs** OMD sheep; **OR** Organic Residue (OR = OM − CP − CL − ST − SU); R^2 coefficient of determination; **RSD** Residual Standard Deviation; **SD** Standard Deviation; **SD%** SD in % of the mean value; **ST** Starch; **SU** Sugar; **UE** Urinary Energy

The accuracy of the *three-stage procedure* is sufficiently high to evaluate the energy value of the feed. However, precisely determined OMD values are available only at a limited number. This limitation is a challenge not only to the *three-stage procedure* described here but also to other approaches for evaluating the energy value of horse feed. In principle, *in vitro* procedures are available for estimating the OMD; however, introducing them into practical feed evaluation requires further standardisation and interlaboratory testing.

For clearly defined feeds with little variation in their composition, it can be assumed that their OMD also varies only within narrow limits. If data for such feeds is available from feed tables of Universität Hohenheim – Dokumentationsstelle (1995), INRA (2015) or from other sources, which differ only slightly from the feed to be estimated, and if the OMD value given has been determined *in vivo*, this can be used as a reliable basis for the ME calculation. However, these tables also contain OMD values for many feeds not based on an *in vivo* determination. The OMD in horses (OMDh) can, however, be derived from the OMD in sheep (OMDs) for several feeds.

The following equation provides a means of **estimating OMDh from OMDs** in forages:

$$\text{OMDh (\%)} = -K + 1.15\ \text{OMDs (\%)}$$

where the following K values are to be used for individual feed groups and the OMDh validity range is to be observed:

K	Feed group	OMDh range (%)
11.5	Lucerne	50–65
12[1]	Maize silage with high proportion of cob	70–80
14.5[2]	Permanent grassland (France)	35–75
17.5[3]	Grassland stand of perennial ryegrass *(Lolium perenne)* (the Netherlands)	45–65
20[4]	Grass stand consisting of timothy *(Phleum pratense)* or cocksfoot *(Dactylis glomerata)*; corresponding grass-rich meadows or grassland listed in the Universität Hohenheim – Dokumentationsstelle (1995, p. 64 and 1997, p. 120); oat and rye whole crops;	50–70
	Cereal straw (including chemically treated)	35–50
22	Grass stand consisting of meadow fescue *(Festuca pratensis)* or tall fescue *(Festuca arundinacea/Lolium arundinaceum)* (Finland)	45–70
10.5[5]	Mixed rations	55–70

[1]Including maize cobs with husks.
[2]The K value given here can only be reliably applied to similar grassland pastures studied by INRA. In addition, note should be made of the comments in Section 3.2.3 on the underlying data, which also suggest a possible method-related cause for the lower K value compared to grass stands.
[3]The K value is not valid for artificially dried grass (Smolders et al. 1990). – A small amount of inconclusive data suggests that a K value of this magnitude could also apply to red clover.
[4]The K value reported here was not derived from experimentally determined differences between OMDs and OMDh of identical feed, but from values in the DLG feed tables for horses and ruminants (Universität Hohenheim – Dokumentationsstelle 1995, 1997).
[5]This group is relatively heterogeneous in terms of composition. The K value given here serves as a rough estimate for mixed rations.

When applying this equation and values for OMDs-OMDh differences, the details given in sections 3.2.3 and 3.3 must be considered. Extrapolations by 5 percentage points (%pt) beyond the given OMDh range are possible (except for rations), but are associated with a somewhat greater degree of uncertainty.

For **sugar and fodder beets, cereal grains** (possibly including oats), mostly for products and co-products of their processing, as well as several **concentrate feeds** that are not described in detail here, if these have an OMD > 80%, a similarly high OMD can generally be assumed for sheep and horses. However, there may also be distinct differences for certain feeds. The energy value of some feeds, therefore, remains uncertain and needs an estimation by an expert. At present, determining the OMD or ME of **compound feeds** is only possible if the proportions of the feed ingredients are known.

The *three-stage procedure* described herein can be used to reliably calculate the ME content of rations and feeds from the OMD. Compared to the procedure used before (GfE 2014), the calculation is more accurate, particularly for protein-rich concentrate feed. Together with the approach presented here of deriving the OMD of many forages of variable composition from the OMD in sheep, and using published *in vivo* determined OMD values of feeds with low variability in nutritional composition, a method is provided for determining the ME content of rations and feeds. In addition, this approach offers a high degree of flexibility and the opportunity to consider new scientific findings and integrate information from other evaluation systems.

1. Background

The Committee for Requirement Standards (AfBN) of the Society of Nutrition Physiology (GfE) has chosen metabolisable energy (ME) as a basis for evaluating the energy content of feed and calculating the energy requirement in horses (GfE 2014). In that system, the ME content of a ration or feed is derived by using an equation for estimating the digestible energy (DE), which is based on the contents of crude protein (CP), crude lipids (CL), crude fibre (CF), and N-free extracts (NfE), and coefficients which are reduced by 8 kJ/g for CP and 2 kJ/g for CF, to account for the urinary energy (UE) loss and CH_4 energy (CH_4-E) loss, respectively. Recently, it has become increasingly obvious that the estimation equation given by the GfE (2014) cannot adequately reflect the differences in the energy value of rations and does not have the accuracy needed for feed evaluation, as is apparent, inter alia, from the studies by Zeyner and Kienzle (2002), Kuchler et al. (2020), Marín et al. (2022), and Pankratz (2022); furthermore, this estimation equation cannot be generally applied to feed ingredients. It was, therefore, necessary to develop a procedure that enables a more accurate ME estimation. This prompted the AfBN to adapt the *three-stage procedure* for determining ME in ruminants (GfE 2023) for horse feeds. This procedure is based on organic matter digestibility (OMD), from which energy digestibility (ED) is derived, with UE and CH_4-E being estimated separately. The principles and advantages of the *three-stage procedure* are described in detail by the GfE (2023) for ruminants and equally apply to horses.

2. Determining the energy digestibility and other energy losses with the *three-stage procedure*

2.1 Energy digestibility

There is a close relationship between OMD and ED, so the content of DE can be derived from the heat of combustion (GE, gross energy) and the OMD. The ED is lower than OMD by a constant value. This difference is, on average, 3.3% percentage points (pt) and 2.8%pt for ruminants and pigs, respectively, and is independent of the feed intake level (FIL) and the OMD level (GfE 2023). A corresponding value for horses was derived based on the following studies:[6] The two trials conducted by Martin-Rosset and Dulphy (1987) on the basis of grass hay and maize silage, with very different concentrate feed (CONC) proportions and FIL, had a mean constant OMD-ED difference of 4.1%pt and 2.1%pt, respectively; the rations had an OMD between 50 and 72% and 71 and 82%, respectively. It is not possible to state with certainty whether such a difference between grass hay-based and maize silage-based rations occurs in general. For the rations having between 52 and 57% OMD that were used by Martin-Rosset et al. (1990) in 6 different animal groups, the mean difference was 3.6%pt. The results from the study by Zeyner et al. (1992) with 9 rations (55–78% OMD) revealed a mean OMD-ED difference of 1.9%pt. In the 8 rations studied by Vermorel et al. (1997a), having between 51 and 65% OMD, the difference was 4.3%pt. In the 14 rations studied by Vermorel et al. (1997b),

[6]The results of the literature review by Sales et al. (2013) could not be considered since the number of studies on which the respective mean value for OMD and ED are based are different and the equation given for estimating ED from OMD has a linear bias between estimated and observed ED values.

having between 45 and 70% OMD, the difference was 4.5%pt. The 9 low-fat rations (59–73% OMD) based on meadow hay and compound feed studied by Zeyner (2002) had a mean difference of 3.9%pt. In Harris et al. (2017), the difference of 8 rations (49–75 % OMD) based exclusively on haylage was, on average, 2.4%pt. The 8 rations (32–79 % OMD) examined by Fehrle (1999) can also be used if it is assumed that the dry matter (DM) digestibility is 1.6%pt lower than the OMD (derived from Martin-Rosset et al. 1990, Vermorel et al. 1997a,b, Harris et al. 2017, Särkijärvi et al. 2012, Saastamoinen et al. 2020, 2021), and exhibit a mean OMD-ED difference of 2.6%pt. If the same assumption is made for the 8 rations (53–80% OMD) from the study by Hipp et al. (2018), the difference is 4.2%pt. This low variation among the OMD-ED differences from the various studies enables the calculation of a mean value, which is 3.4%pt as expressed with Equation [1].

[1] $ED\ (\%) = OMD\ (\%) - 3.4$

The standard deviation (SD) of the mean differences within the studies is less than 1%pt, and between the studies is 1%pt, so an error of < 0.2 MJ/kg DM is to be expected when applying this mean value. This relatively low estimation error clearly demonstrates that ED can be derived with high accuracy from OMD. Using the linear equation given by Martin-Rosset et al. (1994), which is adopted in the INRA system (2015) and describes the dependence of ED on OMD differentiated by CONC and forage, the mean OMD-ED difference derived for forage in the range between 45 and 65% OMD is 3.9%pt and for CONC between 70 and 85% OMD is 2.9%pt, which is in close agreement with the value of 3.4%pt derived for all rations and feeds.[7] The difference does not depend on the magnitude of the OMD. An influence of FIL on this difference was only observed in one of three studies that could be evaluated in this regard, meaning that a general effect cannot be assumed.

The mean OMD-ED difference of 3.4 applies to rations with CL concentrations in the range of up to 4–5% DM. A smaller difference occurs in rations with higher contents of highly digestible fats if the fat-free OM has a distinctly lower digestibility than CL; this situation might occur, for example, when vegetable oil is added to a ration. In such cases, it is appropriate to carry out two separate calculations, one for the ration without added fat and one for the added fat, for which no difference between the digestibility of CL and ED is assumed.[8]

2.2 Urinary energy loss

The energy lost with urine is 3.8–9.4% of GE or 7.0–11.9% of DE (Kienzle and Zeyner 2010) and is thus higher than in ruminants. The variation in UE is predominantly related to the amount of CP intake. An evaluation of the literature and own data by Kuchler et al. (2020) resulted in the following equation, which describes the dependency of the urinary energy losses on the CP concentration of the feed (range 5–24% of DM):

[2] $UE\ (MJ/kg\ DM) = 0.33 + 0.0043\ CP\ (g/kg\ DM)$ $(n = 38;\ RSD = 0.12;\ R2 = 0.81)$

It applies to a metabolic situation where the amount of N entering the metabolism with the digestible CP is predominantly excreted with the urine and no significant body protein loss occurs. More recent data from Pankratz (2022) match the values predicted by equation [2] very well. The UE values given by Vermorel et al. (1997a,b) align with the values estimated according to equation [2]. Rations based exclusively on forage in the range from 93 to 200 g of CP/kg DM (Ragnarsson 2009) do deviate from the estimated value for some rations but not from the mean value, and the deviation did not depend on the CP concentration.[9]

The constant term in the equation [2] reflects the proportion of UE independent of CP intake. This proportion is caused by the obligatory metabolic N losses and the excretion of other energy-rich compounds. Particularly significant here is hippuric acid, produced by the metabolization of phenolic compounds from lignin-carbohydrate complexes of cell walls. Should it be possible in the future to quantitatively describe the influences on UE independent of the CP intake, this could be taken into consideration by modifying the constant term of equation [2].

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[7] The magnitude of the difference in the OMD-ED difference between CONC and forage is therefore relatively small and is within the RSD (residual standard deviation) of this equation given by Martin-Rosset et al. (1994) and the SD of equation [1]; therefore, a corresponding differentiation does not appear to be indicated. It is assumed that a large part of the data on which the equation given by Martin-Rosset et al. (1994) is based originates from the individual studies cited here. However, this equation holds a contradiction in that the OMD-ED differences become smaller as the OMD decreases; such a relationship cannot be confirmed by the available experimental findings. For this reason, this equation is not recommended for use

[8] It should be noted that the OMD value given in tables for pure feed fats is frequently equated with the digestibility of the CL fraction, which, however, fails to take account of endogenous loss; due to endogenous loss, OMD is lower than CL digestibility. If a value for the CL digestibility of pure feed fats is available, this should be used as an approximation for ED, since these two values have a lower difference.

[9] Data from Vermorel et al. (1997a,b) and Ragnarsson (2009) are part of the data set used by Pankratz (2022).

The slope in equation [2] characterises the energy loss per additional gram of CP intake, which is only slightly higher than the value that would be expected if proteins are completely deaminated and the N contained therein is excreted in the form of urea. Therefore, the total loss of UE per g of CP decreases with increasing CP content of the ration. This also ensures that CP-rich feeds, which have CP concentrations above the investigated range and are used as supplements in rations, are not undervalued. The UE values estimated with equation [2] for feeds are additive and, therefore, fulfil the prerequisite for the ration calculation. The RSD of equation [2] with 0.12 MJ/kg DM shows that the UE for the purpose of feed evaluation can be estimated with sufficient accuracy solely from the CP content. It should be noted that UE is applied at the value of 0.33 MJ/kg also for feeds in which no direct urinary energy losses are to be expected, such as vegetable oils or pure starch, since equation [2] was derived from rations containing forage; however, any potential inaccuracies here – possibly also for feeds low in phenolic compounds – are negligible.

2.3 Methane energy loss

According to a literature review by Kienzle and Zeyner (2010), the CH_4-E loss is between 1.9 and 4.2% of GE, or between 3.4 and 6.9% of DE. Thus, it is lower than the UE loss. Methane production is markedly lower in horses than in ruminants, since a considerable proportion of the nutrients not subjected to fermentation are digested in the small intestine. As a result of extensive reductive acetogenesis in the large intestine, less methane is produced per gram of fermented substance than in the forestomach of ruminants. The energy losses via the release of hydrogen (H_2-E) are low compared to CH_4-E and are in the range from 1 to 7% of CH_4-E (derived from data of Zentek et al. 1992, Zeyner 2002, and Mößeler et al. 2005). It is assumed that the measured ratio of the two gas concentrations in the exhaled air reflects the ratio of their total release. The data of Mößeler et al. (2005) showed that the elevated H_2 release that occurs with specific rations was associated with a reduction in CH_4 release, where the reduction in CH_4-E was greater than the increase in H_2-E. The loss of CH_4-E + H_2-E in rations with low H_2 formation was, therefore, higher than in those with high H_2 formation. In the two other studies mentioned, neither such a negative nor a positive interaction was observed. The H_2 formation predominantly depends on the carbohydrate source [cellulose + hemicelluloses (grass pellets) and pectins (dehydrated beet pulp) < starch (oats) < inulin (Jerusalem artichoke)] and appears to be unrelated to CH_4 formation. Since a reliable estimate for H_2 release is currently not possible, and furthermore, the very small proportion of H_2-E is not relevant for the energy evaluation of feed, H_2-E is not considered in the *three-stage procedure*.

The CH_4 production depends on the content of fibre fractions or non-starch polysaccharides. A feeding situation in which relatively large amounts of starch enter the large intestine is not considered here, since such a situation is not regarded as physiological and should be avoided by an adequate ration formulation (GfE 2014). Due to increased fermentation losses, it would not be appropriate to describe the energy supply potential of a feed. A comprehensive literature review (A. Zeyner, C. Böttger, A. Susenbeth, personal communication)[10] showed a mean CH_4-E production of 0.49 MJ/kg DM (SD = 0.11); this is within the range of 0.35 to 0.60 MJ/kg DM for mean values reported in the literature (Kienzle and Zeyner 2010). The CH_4-E/g CF was 1.99 kJ (SD = 0.38) and was not influenced by the CF concentration. There was no correlation between CH_4-E related to the organic residue [OR = OM − CP − CL − ST (starch) − SU (sugar)] and the concentration of OR. Almost all non-starch polysaccharides are captured with the calculated OR. The mean methane energy loss is thus

[3] $CH_4\text{-E} = 0.83$ kJ/g OR (n = 60; SD = 0.16; SD% = 19; range 486–736 OR, g/kg DM)

No effect of the feed intake level on CH_4 release per g of CF or OR was found for the range from 4.7–8.0 kg DM intake/day. If this value is used to estimate the CH_4-E loss for a ration, the resulting error (SD) is approximately 0.10 kJ/g DM. This is lower than when deriving ED from OMD, and similar to the estimate of UE. Thus, the CF concentration of a feed is not required to estimate the CH_4 release.

[10]The data pool was formed by the results of Fingerling's investigations from the years 1931–1939. A total of 60 rations were examined. The basic rations consisted either of meadow hay, oat grains, linseed meal and molasses or only meadow hay. The following feeds were tested in the difference trial: straw of different botanical origin, straw products, meadow hay cut at different times, lucerne and clover hay, oats, flaked maize, rye bran, wheat and rice gluten, fodder beet, sugar, potato flakes, potato starch, faba beans, brewers' grain, cocoa hulls, peanut oil. The contents of ST and SU were derived from the corresponding information in feed value tables based on the feed used and the proportions thereof in the ration. The mean contents, the SD thereof and range were per kg DM for CP 127, 19.0, 102–237, for CL 53, 12.4, 23–107, for CF 250, 41.2, 173–361, for ST + SU 156, 58.7, 66–299, and for OR 596, 62.8, 486–736.

2.4 Influences of feed intake level, breed, and physiological status on organic matter digestibility

Only a few studies mainly attributed to the research group around Martin-Rosset investigated the influence of the feed intake level (FIL) on OMD in horses (Martin-Rosset et al. 1987, Martin-Rosset and Dulphy 1987, Martin-Rosset et al. 1990). The feed intake level 1 (FIL1) was defined by GfE (2023) as 50 g DM/metabolic body size ($kg^{0.75}$)[11] and is adopted herein. The estimation of the effect on OMD in the energy evaluation system according to Vermorel et al. (1997c) and INRA (2015) is based on the results of these investigations. The AfBN essentially adopts the conclusions derived therefrom for the INRA system (2015), in which, however, the possible influence of the FIL on methane production is not addressed. It is assumed that an increase in FIL from 1 to 2–2.5 (i.e. to 100–125 g DM/$kg^{0.75}$ BW) will not result in a reduction in OMD. A FIL of this magnitude is generally not markedly exceeded, even during lactation or in working animals (GfE 2014). The results of Ragnarsson (2009), according to which a lower OMD and ED occurred at a higher FIL, are not to be considered here, since the low FIL in this study was considerably below 1. Furthermore, it is assumed that OMD does not differ between light and heavy breeds; however, ponies have a slightly higher digestibility, by 2 percentage points, compared to light breeds, whereat this value is to be regarded as not constant according to Hoffmann et al. (1987). The assumption of a somewhat higher OMD in ponies is held, although in the study by Potter et al. (2022) this effect did not occur, possibly due to the facts that the faeces were only collected over 1 day, the number of animals was low, and the body weight differences between the breeds were not very great. It is also assumed that digestibility does not differ between weaned young and adult animals (Martin-Rosset and Dulphy 1987, Harris et al. 2017) and between lactating and non-lactating mares despite distinctly different feed intake. However, it is reduced in mares in the 8th to 11th months of gestation. Light to moderate work is assumed to have no impact when rations containing at least 40% forage are fed and at FIL of 2–2.5.

Martin-Rosset and Dulphy (1987) showed, using rations containing grass hay and maize silage, that even at high CONC proportions (up to 60 and 90% of the ration, respectively), the digestibility values were additive and there was no interaction with the FIL; the digestibility of the forage was unrelated to its proportion in the ration, in contrast to observations from digestibility trials concurrently conducted with sheep.

To conclude, current knowledge indicates that the FIL, the ration composition, and the physiological status of the horse (with the exception of late gestation) do not need to be considered in feed evaluation as factors affecting OMD. No studies or considerations on the possible effects of these factors on methane production were found in the literature. Because of the relatively low methane production, such influences would have little quantitative significance.

3. Determining gross energy and organic matter digestibility

3.1 Determination of the heat of combustion

The heat of combustion of the feed represents its gross energy (GE) and is to be determined by calorimetry (DIN, 2003). If a calorimetric determination is not possible, GE can be calculated by using the following equation (Hoffmann et al. 1993, Beyer et al. 2003):

[4] GE (kJ/kg DM) = 23.6 CP + 39.8 CL + 17.3 ST + 16.0 SU + 18.9 OR (in g/kg DM)

where CP, CL, ST, SU and OR are crude protein, crude lipid, starch, sugar, and organic residue[12]. Equation [4] gives reliable values for rations and most feed ingredients. Although it is associated with inaccuracies in some instances (GfE 2023), these are considered to be of minor relevance for most feeds, compared to using approaches with greater error sources, e.g. the determination of OMD in horses (OMDh) from OMD in sheep (OMDs; see Section 3.2.3). The estimation equation given by the GfE (1995) (adopted from Hoffman et al. 1971) and based on the concentrations of

[11]The FIL1 is not the same as feeding level 1. However, if an OMD for the ration is 65%, this corresponds to an energy supply in the range of the maintenance requirement, if 0.50 MJ ME/kg0.75 BW is assumed for this. This roughly matches the value of 32 g digestible OM/kg0.75 BW, at which, according to Jarrige and Martin-Rosset (1984) (cited by Martin-Rosset et al. 1990), the energy maintenance requirement is covered. FIL is also often defined as DM intake in % of BW. For a BW of 600 to 650 kg, both references give roughly the same value (FIL1 = 6.1 or 6.4 kg DM).

[12]The various methods for determining ST and SU sometimes lead to significant differences in content, which consequently lead to corresponding differences in the OR content. The GE contents calculated according to this equation are however only affected to a relatively low extent thereby, since the coefficients for ST, SU and OR do not differ very greatly and the differences in the analysed content therefore only have an effect corresponding to the difference in their coefficients. For certain straight feeds, the notes accompanying the respective methods of the VDLUFA (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten) (2012) should be borne in mind.

CP, CL, CF, and N-free extract can provide reasonable mean estimates of the calorimetrically determined GE within the range of 18.0–18.5 MJ/kg DM (Pankratz 2022). However, this equation showed considerable variation and systematic deviations at lower and higher GE values; therefore, its use is not recommended.

3.2 Determination of OMD

3.2.1 Digestibility trials

The effort in determining OMD or ED in digestibility trials is high and the number of published, *in vivo*-determined OMD values is limited. These OMD values are particularly useful in ration planning in the case of feeds with only small variations in their chemical composition and digestibility that can be clearly assigned to a well-defined feed group. As outlined in the following sections, the errors that occur when estimating OMD using *in vitro* assays or from OMD in sheep and the uncertainty for a considerable number of feeds is, in parts, high. This underlines the importance and necessity of digestibility trials in horses because their accuracy and reliability cannot be achieved by other methods. Digestibility measurements in animals are, therefore, an indispensable reference, in particular as a basis for developing and testing alternatives to the digestibility trial.

3.2.2 Estimating OMD with *in vitro* methods

In vitro methods have gained great importance in the field of ruminant nutrition. They make it possible to estimate OMD with sufficient accuracy (GfE 2023). Depending on the feed group, an RSD between 2 and 5%pt is to be expected. The suitability of *in vitro* methods for horses has been investigated in a series of studies and estimation equations have been published. The AfBN used the studies by Smolders et al. (1990), Macheboeuf et al. (1998a/b), Zeyner and Dittrich (2005), Martin-Rosset et al. (2012), and INRA (2015) as a basis for evaluating their suitability in practical feed evaluation. When using these studies, it should be noted that similarly named *in vitro* methods are not always identical. Therefore, when using estimation equations, the assay details must be carefully observed.

The AfBN agree with the view of Martin-Rosset et al. (2012) that *in vitro* methods are, in principle, suitable for horse feed evaluations. The estimation equation developed by Martin-Rosset et al. (2012) for forage (RSD = 2.0) and the equation given by INRA (2015) for CONC ("processed ingredients"; RSD = 5.6), which are based on enzymatic OM digestion *in vitro*, as well as results provided by Macheboeuf et al. (1998a/b) for grassland and lucerne, which are based on the 24-h *in vitro* gas production with caecal content (RSD = 2.7 or 2.2) or horse faeces (RSD = 2.8 or 2.5) as inocula, can serve as suitable methods for practical application if standardisation, validation and evaluation by inter-laboratory tests have been carried out beforehand. Hence, *in vitro* methods have great potential for reliable OMD determination in future.

An opportunity to overcome the huge effort of digestibility trials for creating reference values for a large number of feeds, which is necessary for developing and testing an *in vitro* method, could be to test only a small number of forages, concentrates, and compound feeds in a digestibility trial and combine it with an established *in vitro* procedure, in order to use these feeds as standards. This enables the calculation of correction factors from the measured gas production and the enzyme-soluble OM of these standard feeds. However, a considerable proportion of the residual variation in regression equations for estimating OMDh might also be due to the variation in the values determined *in vivo*, since the variation of the OMD values is distinctly higher in horses and ponies than in sheep (Hoffmann et al. 1987), which necessitates an adequate number of animals and duration of the faecal collection period.[13]

3.2.3 Estimation of OMD in horses from OMD in sheep

OMD in horses (OMDh) is related to OMD in sheep (OMDs) since the digestive systems of both species are able to degrade large amounts of non-starch-polysaccharides. This indicates the opportunity to derive OMDh from OMDs, which entails the advantage that comprehensive data from digestibility studies in sheep can be used, whereas results from digestibility trials in horses are only available to a limited number. In general, the OMDh of forages and rations is lower than the OMDs. The difference between OMDh and OMDs is greater in fibre-rich forage, decreases with increasing OMD, and does not generally occur in highly digestible feeds or rations. Thus, Särkijärvi et al. (2012)

[13]Goachet et al. (2012) pointed out poor repeatability of OMD values in their trials, possibly due to faecal collection periods that were too short. Zeyner (2005) provides information on how to conduct digestibility studies, and in addition advises a collection period of at least 5 days. If a marker is used, its recovery and SD must be determined. – The physiological causes for the relatively large variance in the digestibility should be investigated in order to possibly take them into account in the digestibility trial. – The need for a certain degree of physical activity of the animals in digestibility trials was pointed out early on (Langworthy 1903).

showed that in grass silages, the digestibility of the neutral detergent fibre (NDF)-free OM was not different, but the NDF-digestibility was significantly lower in horses than in sheep, and the difference became smaller as NDF digestibility increased. Langworthy (1903), Fingerling (1940, cited by Axelsson 1941), and Axelsson (1941) had already pointed out that the difference in the digestibility is most pronounced for CF compared to other crude nutrients, albeit not consistently. Thus, the OMDs-OMDh difference essentially depends on the concentration and digestibility of the fibre fractions. However, this difference can be specific for certain feed groups, which cannot be attributed to the fibre content or the digestibility. Therefore, generalising the relationship between OMDh and OMDs is not possible.

The following proposal for estimating OMDh from OMDs is based on the studies by Martin-Rosset et al. (1984), Schmolders et al. (1990), and Särkijärvi et al. (2012) as well as an equation given by Zeyner et al. (1994).[14] Besides the estimation equation for rations (Zeyner et al. 1994), the other studies provided differentiated estimation equations for certain feed groups. However, their definitions are not always precise enough to assign a specific feed to a certain feed group, making a comparison between the equations difficult and preventing an agreement or the identification of deviations among the studies. The various linear regression equations in the form of OMDh (%) = b_0 + b_1 OMDs (%) were tested and evaluated with regard to the underlying data and the plausibility of their function values. Equations were classified as implausible if they resulted in an increasing difference between OMDh and OMDs with increasing OMD within a feed group or if they had a distinctly higher OMD in horses than in sheep within the range of average digestibility.

Four of these equations,[15] which fulfil these test criteria, show very similar b1 values, although the various feed groups had variable differences between OMDh and OMDs, which are mainly reflected by the b0 value. Therefore, the calculation of a mean b1 value for general use is appropriate. The b1 value indicates the decrease of the difference between OMDs and OMDh (%pt) if OMDs increases by 1%pt. In this way, a generalisation can be achieved if the difference between various feeds is considered by using feed group-specific b0 values (given here as K value). OMDh can be approximated from OMDs for some forage groups and rations as follows:

[5] OMDh (%) = −K + 1.15 OMDs (%)

with the following K values for feed groups:

The difference between OMDs and OMDh for rations within the investigated ranges is between 2.0 and 0. At appro-

K	Feed group	derived from the OMDh range (%)
11.5	Lucerne[16]	51–62
14.5	Grassland pasture[17]	36–76
17.5	grass stand of perennial ryegrass *(Lolium perenne)*[18]	46–65
22	grass stand[19] of meadow fescue *(Festuca pratensis)* or tall fescue *(Festuca arundinacea)*	46–67
10.5	Mixed rations[20]	55–70

[14]In the given equation, the designation of the dependent and independent variables has seemingly been swapped, which was taken into consideration here.
[15]These are equations for the following feed groups: grassland pasture and lucerne (Martin-Rosset et al. 1984), grass stand of perennial ryegrass (Smolders et al. 1990), grass stand consisting mainly of meadow fescue and tall fescue (Särkijärvi et al. 2012).
[16]In the publication on which the present report is based (Martin-Rosset et al. 1984), the more comprehensive designation 'legumes' is used, although the majority of the data collected was for lucerne and only 20% was made up of other forage legumes. The restriction to lucerne is also based on the good correlation with values from the DLG feed value tables (Universität Hohenheim – Dokumentationsstelle 1995 and 1997; see Section 3.3).
[17]In the publication on which the present report is based (Martin-Rosset et al. 1984), the more comprehensive designation 'grassland pasture and grass stand' is used, although only around 15% of the data collected were for grass stands and 10% for straw rations. The studies were conducted in France prior to the year 1984. The K value given here can only be confidently applied to similar grassland pastures studied by INRA. A considerable amount of literature data for grassland pasture was excluded by the authors from the evaluation on the grounds that they display significantly larger OMDs-OMDh differences and originate from studies that date back a long time and were carried out on working horses; these data lead to a K value of approx. 21.5.
[18]The K value is not valid for artificially dried grass (Smolders et al. 1990).
[19]One of the 6 rations investigated here consisted of Timothy grass (Phleum pratense). – The study with Timothy grass by Udén and Van Soest (1982) was not considered, since only two animals were used and the digestibility thereof differed significantly.
[20]This group is more heterogeneous in terms of composition compared to the others and thus also displays a greater residual variance. The experiments by Hoffman et al. (1987) show that at high OMD, OMDh can also be above OMDs; a K value for rations of a similar order of magnitude to that stated the above can likewise be derived from these experiments.

ximately 70% OMD, horses and sheep exert similar OMD values, which is supported by the results of Martin-Rosset and Dulphy (1987) and Hoffmann et al. (1987) using rations consisting mainly of grass hay and relatively high proportions of CONC. A very low difference also results from the two equations given by Axelsson (1941) when the OMD exceeds 70%. [21] An application to rations >70% OMD is not feasible.[22] For lucerne, the difference is between 3 and 2, for grassland between 8 and 3, for grass stands of perennial ryegrass between 9 and 7, and those of meadow fescue or tall fescue between 13 and 10%pt for the specified OMDh ranges. Based on the original equations, the accuracy of the estimate for lucerne, grassland pastures and grass stands, expressed as RSD, is assumed to be in the range from 1 to 2.5, and 5.5 for rations.[23] Equation [5][24] consistently reflects the relationships between OMDs and OMDh described by the above-mentioned four equations from the literature.

When using the K values given for equation [5], it should be noted that these are only valid for the material on which the studies are based, which should be considered given the heterogeneity of grassland pastures, grass stands, and rations to be evaluated. Therefore, a reliable feed assignment to any of the groups is not always possible. It also cannot be ruled out that the differences in values of grassland pasture and grass stands were caused not only by the botanical composition but also by location factors and the research methodology. The K value is derived from studies conducted for grassland pasture and lucerne in France[25], for grass stands of perennial ryegrass in the Netherlands, and for other grass stands in Finland. According to Müller (2012), the type of preservation (hay, silage) does not affect OMD; moreover, the OMDs-OMDh difference between hay and fresh herbage was not different (Martin-Rosset et al. 1984; Smolders et al. 1990). Mechanical processing of dried lucerne (long particles, chopped, pressed, pelleted) did not affect DM digestibility (DMD) and ED (Todd et al. 1995). Therefore, the relationship described with equation [5] is considered unaffected by preservation and particle size at this time.

No OMD-dependent differences occur for feed groups other than the aforementioned, and possibly also for feed ingredients, but mean differences do occur, albeit very inconsistently. According to Schmolders et al. (1990), the average difference between OMDs and OMDh was 2%pt (SD = 4) for some products and co-products of milling industries and 4%pt (SD = 6) for cereal grains and grain legumes; for compound feeds, OMDh was 3.5%pt (SD < 2) higher than OMDs.[26] The SD values reflect the relatively large inconsistency of this difference; also, the data basis with three feeds per feed group was low. Thus, these values and the differences between these feed groups cannot be generalised.

Equation [5] provides a framework to estimate OMDh from OMDs for the above-mentioned forages together with the proven OMDs-OMDh differences for other forages and some CONC (see Section 3.3). For other feeds, a higher estimation error and a greater degree of uncertainty are to be expected, which are likely to increase if feed ingredients cannot be precisely assigned or if an extrapolation is necessary that goes beyond the analysed data range. Although the differentiation by feed groups made here allows for a sufficiently reliable estimate for some forage types in practice, no appropriate data basis is available for other forages (e.g. forage legumes). An improvement in the respective estimation accuracy can be achieved if, in addition to OMDs, the NDF concentration is considered a variable (Smolders et al. 1990). However, the AfBN does not consider corresponding equations to be reliable enough for general application.

Regarding the accuracy in estimating OMDh from OMDs, it should also be mentioned that estimation equations for the calculation of OMD in ruminants using *in vitro* data likewise have RSD values between 2 and 3, and in some cases also up to 5%pt (GfE 2023). An error of the same order of magnitude is to be expected for *in vitro* methods in horses (Smolders et al. 1990). Thus, the approach described here of deriving OMDh from OMDs is advantageous because a comprehensive amount of data is available on ruminants and *in vitro* methods are established. However, the further calculation step increases the error associated with estimating OMDh from OMDs, when determined with an *in vitro*

[21] Axelsson (1941) gives a linear equation for horses and cattle in each case, which describes the influence of the CF concentration on the OMD.
[22] Although it has been observed that in rations with a low proportion of forage, OMDh was also above OMDs (Hoffman et al. 1987), this cannot be generalised. Conversely, an extrapolation to higher OMD values by 5%pt should still lead to reliable values for forage.
[23] When assessing the SD, which occurs when estimating OMDh from OMDs, it should be noted that the determination of the respective OMD can also be subject to a not insignificant error. It can therefore be assumed that the actual mean deviation between OMDh and OMDs is lower and the estimation accuracy is higher.
[24] A model that is similar in principle has already been proposed by Smolders et al. (1990) for grass preserved in various ways.
[25] Clarification is needed here: the equation for grassland pastures given by Martin-Rosset et al. (1984) and often referred to in the literature is based exclusively on data compiled by INRA, whereas the equations for forage legumes also used data from the literature on lucerne and red clover. According to Smolders et al. (1990), the smaller OMDs-OMDh difference for these grassland pastures compared to their own results with perennial ryegrass could be due to the higher FIL thereof in sheep. If this were the case, a suitably adjusted K value would differ only slightly from the value for the perennial ryegrass grass stand.
[26] The authors could not provide an explanation; grinding and pelleting are ruled out as a cause since, according to several sources, they do not influence digestibility.

approach.[27] It should also be noted that, in contrast to sheep, the FIL in horses up to a certain FIL has no effect on OMD (see Section 2.4). This needs to be considered when using results from digestibility studies in sheep that deviated from the FIL recommended by GfE (1991).[28]

The OMDh value derived from OMDs is regarded as reliable, particularly if the objective is to establish differences in OMDh within feed groups. Nevertheless, considerable uncertainties remain for a number of concentrates and forages with regard to their OMDh value. Future efforts should be directed at developing generally valid estimation procedures for other, preferably large feed groups, which may be achieved by considering various chemical fractions.

3.2.4 Estimation of OMD from fibre fractions

The OMD is largely determined by the concentration of fibre fractions in the feed. The digestibility of the fibre fractions is determined, in particular, by the degree of their lignification. Other nutrients or nutrient groups commonly have a higher and less variable digestibility. The effect of the 'cell wall' on the digestibility of the feed has been investigated early on using CF (Axelsson 1941, Olsson 1949). Due to its worldwide use in feed analysis, CF is of particular importance. It is still analysed to date to characterise feeds, although the limitations of the CF assay have been known for a long time. The number of data from metabolic studies in horses, in which the fibre content is described using the CF, is extensive. Knowing the CF content is a prerequisite for calculating both the ME of a feed and the ME utilisation in the INRA system (2015).

A series of equations have been derived for a possible application to feed evaluation with a large data set representing large feed groups (forages, concentrates) or rations. They describe the dependence of OMD on CF concentration (Axelsson 1941; Olsson 1949; Martin-Rosset et al. 1984, cited by Martin-Rosset et al. 1994 and INRA 2015; Schulze 1987; Smolders et al. 1990; Zeyner 1995; Fehrle 1999; INRA 2015). These equations can be used for OMD estimation and have an RSD between 2 and 7% units.[29] However, some equations vary considerably regarding the regression coefficient, which indicates how much OMD is influenced by the CF concentration. This is not surprising, since the fibre fraction has a very heterogeneous composition and varies greatly in the degree of lignification. The lignification affects the digestibility of the fibre fraction to varying degrees in different feeds, and the CF only covers part of the cell wall carbohydrates and lignin in the feed – and, moreover, a variable one. In some equations, particularly those derived from cereal grains and cereal co-products, the regression coefficient of less than −1 indicates that the CF itself has a low digestibility and the digestibility of other nutrients may be affected by the fibre fraction. An improvement in the estimation accuracy of the OMD from the CF was achieved when equations were calculated for narrowly defined feed groups or further chemical fractions were considered, although these were sometimes based on a relatively small data set.

Because of the method-inherent insufficient differentiation of the fibre fractions in the CF analysis, the relatively large variation in the relationship between OMD and CF concentration, and the partly considerable differences in the published equations describing this relationship, it can be inferred that CF can be used as an approximation to differentiate OMD and the energy value of feed, in particular within a certain feed group. However, the influence on the OMD of a feed is not reflected by the CF with an accuracy required for feed evaluation. Smolders et al. (1990) showed that for forages, the CF concentration and for CONC, the NDF concentration were the better variables for estimating OMD. In Martin-Rosset et al. (2012), estimating the OMD of forages from the NDF or acid detergent fibre (ADF) concentration did not result in a higher estimation accuracy compared to the CF concentration, whereas the estimation from the acid detergent lignin (ADL) concentration was distinct less accurate. A literature review by Sales et al. (2013) suggested that for forages, the NDF concentration is superior to the ADF or ADL concentration in terms of its estimation accuracy. It can be concluded from these studies that estimating the OMD from fibre fractions as the sole variable, irrespective of the determination method, is not possible with sufficient accuracy; furthermore, the various equations do not lead to consistent OMD estimates. However, taking other predictor variables into consideration did not generally increase the accuracy of the equations developed for certain feed groups. Although the accuracy is expected to increase the more narrowly a feed group is defined, the more such equations suffer from the restriction to their scope of validity, particularly if feeds or feed mixtures have a different composition or cannot be assigned.

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[27] If the causes of the variation and error are unrelated, the error when applying both steps results from the square root of the sum of the individual variances.
[28] This is pointed out by Smolders et al. (1990) who consider a different FIL in sheep to be a possible cause for findings that are not consistent with Chenost et al. (1985).
[29] For the equations from Axelsson and Olsson, the RSD values are not known.

Relevant results from a recent study, which was based on the most comprehensive pool of data to date are presented in the following (M. Coenen, personal communication). A total of 971 OMD values for rations and feed ingredients, based on an average of 5.9 individual measurements per ration or feed ingredient, were taken from 250 publications from 1881 to 2024. The concentrations of NDF, ADF, and CF were not known for all rations; thus, the calculated regression equations were based on the same rations in the majority, but not completely. The means and ranges for OMD were 61.3% (24–99; n = 971[30]), for the concentrations of CP 125 (7–556; n = 967), NDF 532 (30–860; n = 683), ADF 315 (35–596; n = 620), and CF 240 (10–478; n = 538) g/kg DM. The linear regression equations between OMD (%) as dependent variable and NDF, ADF, and CF (g/kg DM) as independent variables were:

$$OMD = 90.2 - 0.057\ NDF \quad (R^2 = 0.48, RSD = 8.0, n = 681)$$

$$OMD = 83.0 - 0.076\ ADF \quad (R^2 = 0.37, RSD = 8.4, n = 618)$$

$$OMD = 88.4 - 0.105\ CF \quad (R^2 = 0.62, RSD = 8.8, n = 537)$$

Simultaneous consideration of NDF and CP as independent variables reflected the negative effect of NDF and the positive effect of CP: $OMD = 85.6 - 0.053\ NDF + 0.022\ CP$ ($R^2 = 0.48$, RSD = 8.0; n=581), but did not lead to a more accurate estimation. Restricting the data to forages (fresh grass, hay, haylage, straw; n = 410) while simultaneously considering NDF and ADF also did not lead to a clear reduction in RSD (7.4, n = 410). Similarly, restricting the data to fresh grass and hay with the independent variables NDF and CP did not cause a marked increase in estimation accuracy: $OMD = 75.3 - 0.042\ NDF + 0.036\ CP$ ($R^2 = 0.65$, RSD = 6.6, n = 231).

This is in contrast to the markedly higher R^2 values of recent equations for estimating OMD by Martin-Rosset et al. (2024), which were calculated separately for hay from grassland pasture, grass stands, and lucerne, but were based on a smaller data set and can be assumed to be probably specific for their location of origin. These have R^2 and RSD values of 0.68 – 0.87 and 2.4 – 3.5%pt, respectively, for grassland pasture and grass stands. If the estimation equation proposed by Martin-Rosset et al. (2024): $OMD = 110.8 - 0.101\ NDF + 0.028\ CP$ ($R^2 = 0.86$, RSD = 2.4, n = 32) is applied to the data for grass and hay mentioned above, the relationship between observed and estimated values for OMD has an $R^2 = 0.42$ and an RSD = 8.9 (n = 231) (M. Coenen, personal communication). Therefore, due to the restricted number of data, the equations given by Martin-Rosset et al. (2024), when applied generally to forage feed of different origins, cannot estimate OMD with sufficient accuracy. Furthermore, it should be noted that in terms of the different sources of this data, it should be assumed that a part of the variance in the relationship between fibre fractions and OMD is caused by differences in the trial and analysis methodologies.

For the sake of completeness, a concept discussed in the literature is addressed here. As explained by Van Soest (1987, chap. 22.3) and adopted by Zeyner (1995), correlations between so-called uniform, chemically analysed properties and digestibility are used as a basis rather than statistical relationships. A distinction was essentially made here between the cell contents, for which a high and constant (uniform) digestibility is known, and the cell wall, which has a variable digestibility due to the degree of lignification. A crucial prerequisite for this approach would be that the effect of lignin can be shown with sufficient accuracy to be a uniform effect, i.e. unrelated to its concentration and origin, on the indigestible portion of the cell wall. Whether such a concept can be a future approach to OMD estimation remains to be clarified.

The results and findings presented in this section confirm, on the one hand, that OMD is, above all, determined by the content of plant cell wall constituents. On the other hand, however, they also show that the common procedures for the analysis of those constituents that reduce digestibility, cannot accurately reflect their effects on digestibility. Hence, a reliable estimate in the scientific sense is not provided and cannot be expected in the future. The processes of organic matter digestion are so complex that, from a current perspective, they can only be quantified in a digestibility trial or by simulating digestion and fermentation.

[30]For part of the data, OMD was derived from DMD.

3.3 The use of data on organic matter digestibility from feed tables

In the feed tables published by Universität Hohenheim – Dokumentationsstelle in 1995 and Martin-Rosset in 2015 (INRA 2015), there is extensive information on the OMD of feeds. In the DLG feed tables (Universität Hohenheim – Dokumentationsstelle 1995), only a small portion of this information originates from digestibility trials; instead, it was mainly taken from very similar feeds or estimated with the aid of regression equations. In INRA (2015), the OMD of forages is estimated from the OMD in sheep, whereas the OMD of CONC was determined in digestibility trials. Both feed tables provide extensive information on OMD, from which the ME of feeds can be derived using the *three-stage procedure*. Feed tables, therefore, are currently an important basis for formulation rations. However, only those OMD values which have been determined in digestibility trials can be considered reliable.

If feeds are not listed in tables, if there is markedly different information on OMD, if it is unclear whether the feed to be estimated corresponds to the feed listed in the tables, or if the accuracy of the OMD value given is in doubt, and if this is an estimation rather than a measurement, it is recommended to use corresponding OMD values for ruminants (e.g. Universität Hohenheim – Dokumentationsstelle 1997) or to determine OMD with the aid of an *in vitro* procedure for ruminants (GfE 2023) (see Section 3.2.3). For grassland pasture, grass stands, and lucerne, it is appropriate to use equation [5] to estimate OMDh from OMDs. The OMDs-OMDh differences calculated according to equation [5] for lucerne hay at various phenological stages are in good agreement with those which can be derived from the respective OMD values in the feed table for ruminants (Universität Hohenheim – Dokumentationsstelle 1997) and those for horses (Universität Hohenheim – Dokumentationsstelle 1995), which supports the reliability of equation [5] for this feed group. Fingerling (1940, cited by Axelsson 1941) had already pointed out that the difference in forage legumes is smaller than in "meadow feed". For grass stands of cocksfoot, timothy, grass-rich meadows (as listed in Universität Hohenheim – Dokumentationsstelle (1995), p. 64[31]), for oats and rye whole crops as well as cereal straw (including chemically treated), mean K values in the range from 19.6 to 21.4 (mean 20.2) can be derived from the respective table values. In general, when making such comparisons (only values measured in digestibility trials were used), it must be considered that the feed materials are not identical, but only have a (largely) corresponding designation, which can cause distinct deviations. For maize silage with an OMD > 70% and a relatively high cob content and starch concentrations > 25%, a K value of 12 can be derived based on only a few data. For fodder beet and sugar beet, cereal grains, and concentrate feeds having an OMD > 80%, in many cases a similar OMD can be assumed in horses and sheep, which is supported by the study of Martin-Rosset and Dulphy (1987) with a highly digestible CONC mixture. An exception is oats, which has a lower OMD. However, for products and co-products from cereal grain and oilseed processing with a lower OMD and for grain legumes, there are variable values for the OMDs-OMDh differences, meaning that for these feeds their OMDs value cannot be used to directly estimate OMDh.

[31]The OMDh values given by the University of Universität Hohenheim – Dokumentationsstelle (1995) for grass-rich meadows were compared with the OMDs values given by the Universität Hohenheim – Dokumentationsstelle (1997, p. 120) for grass-rich grassland with the same CF contents.

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Das *dreistufige Verfahren* zur Bestimmung der Umsetzbaren Energie von Futtermitteln für Pferde

Empfohlene Zitierweise:

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Zusammenfassende Übersicht:

Anwendung des *dreistufigen Verfahrens* zur Bestimmung des Gehalts an Umsetzbarer Energie

Nachfolgend sind die für eine Anwendung des Verfahrens relevanten Schritte und Aspekte zusammengefasst. Die sich anschließenden nummerierten Abschnitte beinhalten detaillierte Erläuterungen zur Vorgehensweise, zur Bewertung und zu den Perspektiven.

Das ***dreistufige Verfahren*** zur Bestimmung des energetischen Futterwerts auf der Stufe der Umsetzbaren Energy (ME, metabolisable energy) besteht darin, dass die bei Pferden auftretenden stofflichen Energieverluste über Kot, Harn und CH_4 jeweils gesondert ermittelt werden. Dadurch weist dieses Verfahren eine klare Struktur auf und ermöglicht eine Weiterentwicklung und Präzisierung in Einzelbereichen. Die Verdaulichkeit der Energie (ED, energy digestibility), die Energieverluste über den Harn (UE, urinary energy) und über die Methanabgabe (CH_4-E) werden wie folgt berechnet:

$$ED\ (\%) = OMD\ (\%) - 3{,}4$$

$$UE\ (MJ/kg\ DM) = 0{,}33 + 0{,}0043\ CP\ (g/kg\ DM)$$

$$CH_4\text{-}E = 0{,}83\ kJ/g\ OR$$

wobei DM (dry matter) Trockenmasse, CP (crude protein) Rohprotein und OR organischer Rest (= OM – CP – CL – ST – ZU) bedeuten. Demnach ergibt sich der ME-Gehalt eines Futtermittels oder einer Ration für Pferde nach folgender Formel:

$$ME\ (MJ) = GE\ (MJ) \cdot [OMD\ (\%) - 3{,}4] \div 100 - [0{,}33 + 0{,}0043\ CP\ (g)] - 0{,}00083\ OR\ (g)$$

(alle Konzentrationsangaben sind auf 1 kg DM bezogen)

Es wird empfohlen, den Brennwert (GE, gross energy) mittels Kalorimetrie zu bestimmen. Es besteht jedoch auch die Möglichkeit, die GE vieler Futtermittel aus den Nährstoffgehalten zuverlässig zu schätzen. **Dieses *dreistufige Verfahren* ersetzt das von der GfE (2014) beschriebene Vorgehen** zur Ermittlung des ME-Gehalts von Futtermitteln und Rationen auf der Basis der Gehalte an Rohprotein, Rohfett, Rohfaser und N-freien Extraktstoffen.

Abkürzungen: **ADF** Acid Detergent Fibre (Säure-Detergenzien-Faser); **AfBN** Ausschuss für Bedarfsnormen; **CA** Crude Ash (Rohasche); **CF** Crude Fibre (Rohfaser); **CP** Crude Protein (Rohprotein); **CH_4-E** Methanenergie; **CL** Crude Lipid (Rohfett); **DE** Digestible Energy (Verdauliche Energie); **DM** Dry Matter (Trockenmasse); **DMD** Dry Matter Digestibility (Verdaulichkeit der Trockenmasse); **DOM** Digestible Organic Matter (Verdauliche Organische Masse); **ED** Energy Digestibility (Verdaulichkeit der Energie); **FAN** Futteraufnahmeniveau (FAN1 = 50 g DM/kg0,75 KM; FANx = x · FAN1); **GE** Gross Energy (Bruttoenergie); **GfE** Gesellschaft für Ernährungsphysiologie; **H_2-E** Energieverluste über Wasserstoffabgabe; **KF** Konzentratfutter; **KM** Körpermasse; **ME** Metabolisable Energy (Umsetzbare Energie); **NDF** Neutral Detergent Fibre (Neutral-Detergenzien-Faser); **NfE** N-freie Extraktstoffe; **OM** Organic Matter (Organische Masse); **OMD** Organic Matter Digestibility (Verdaulichkeit der Organischen Masse); **OMDh** OMD Pferd; **OMDs** OMD Schaf; **OR** Organic Residue (Organischer Rest; OR = OM − CP − CL − ST − ZU); **R^2** Bestimmtheitsmaß; **RSD** Residual Standard Deviation (Reststreuung); **SD** Standard Deviation (Standardabweichung); **SD%** SD in % des Mittelwerts; **ST** Stärke; **UE** Urinary Energy (Harnenergie); **ZU** Zucker

Die Genauigkeit dieses neuen Verfahrens ist für den Zweck der energetischen Futterbewertung ausreichend hoch. Präzise bestimmte OMD-Werte liegen jedoch nicht für alle bei Pferden genutzte Futtermittel im erforderlichen Umfang vor. Hiervon sind jedoch neben dem hier beschriebenen *dreistufigen Verfahren* auch andere Systeme der energetischen Bewertung von Futtermitteln für Pferde betroffen. Zwar stehen grundsätzlich geeignete *in-vitro*-Verfahren zur Schätzung der OMD zur Verfügung, deren Einführung in die praktische Futterbewertung aber weitere Standardisierungen und Ringuntersuchungen voraussetzt.

Für eindeutig definierte Futtermittel, welche nur eine geringe Streuung in ihrer Zusammensetzung aufweisen, kann unterstellt werden, dass auch deren OMD nur in engen Grenzen variiert. Liegen für solche Futtermittel Angaben aus Futterwerttabellen der Universität Hohenheim – Dokumentationsstelle (1995), des INRA (2015) oder aus anderen Quellen vor, die sich nur wenig von dem einzuschätzenden Futtermittel unterscheiden, und ist der angegebene OMD-Wert in einem Verdaulichkeitsversuch bestimmt, kann dieser als zuverlässige Grundlage für die ME-Berechnung herangezogen werden. In diesen Tabellen sind allerdings auch für viele Futtermittel OMD-Werte angegeben, die nicht auf einer *in-vivo*-Bestimmung beruhen. Die OMD beim Pferd (OMDh) kann jedoch für eine Reihe von Futtermitteln aus der OMD beim Schaf (OMDs) abgeleitet werden.

Eine Möglichkeit zur **Einschätzung der OMDh aus der OMDs bei Grobfuttermitteln** bietet folgende Gleichung:

$$\text{OMDh (\%)} = -K + 1{,}15\ \text{OMDs (\%)}$$

wobei für einzelne Futtermittelgruppen folgende K-Werte einzusetzen sind und der OMDh-Gültigkeitsbereich zu beachten ist:

K	Futtermittelgruppe	OMDh-Bereich (%)
11,5	Luzerne	50–65
12[1]	Maissilage mit hohem Kolbenanteil	70–80
14,5[2]	Dauergrünlandaufwuchs (Frankreich)	35–75
17,5[3]	Grasbestand aus Deutschem Weidelgras *(Lolium perenne)* (Niederlande)	45–65
20[4]	Grasbestand aus Wiesenlieschgras *(Phleum pratense)* oder Knaulgras *(Dactylis glomerata)*; entsprechende bei Universität Hohenheim – Dokumentationsstelle (1995, S. 64 und 1997, S. 120) aufgeführte grasreiche Wiesen bzw. Grünland; Hafer- und Roggenganzpflanzen;	50–70
	Getreidestroh (auch aufgeschlossenes)	35–50
22	Grasbestand aus Wiesenschwingel *(Festuca pratensis)* oder Rohrschwingel *(Festuca arundinacea/Lolium arundinaceum)* (Finnland)	45–70
10,5[5]	Gemischte Rationen	55–70

[1]Einschließlich Maiskolben mit Hüllblättern.
[2]Der hier angegeben K-Wert ist nur für ähnliche wie von INRA untersuchte Grünlandaufwüchse sicher anzuwenden. Außerdem sind die Anmerkungen zu den zugrunde liegenden Daten im Abschnitt 3.2.3 zu beachten, die auch einen Hinweis auf eine mögliche methodisch bedingte Ursache für den gegenüber Grasbeständen geringeren K-Wert enthalten..
[3]Der K-Wert gilt nicht für künstlich getrocknetes Gras (Smolders et al. 1990). – Wenige und unsichere Daten deuten darauf hin, dass ein K-Wert in dieser Größenordnung auch für Rotklee zutreffend sein könnte.
[4]Der hier angegebene K-Wert wurde nicht aus experimentell bestimmten Unterschieden zwischen OMDs und OMDh identischer Futtermittel abgeleitet, sondern aus Werten der DLG-Futterwerttabellen für Pferde und Wiederkäuer (Universität Hohenheim – Dokumentationsstelle 1995, 1997).
[5]Diese Gruppe weist eine relativ große Heterogenität in der Zusammensetzung auf. Der hier angegebene K-Wert dient zur überschlägigen Einschätzung gemischter Rationen.

Bei Anwendung dieser Gleichung, d. h. Nutzung der OMDs-OMDh-Differenzen, sind die näheren Ausführungen in den Abschnitten 3.2.3 und 3.3 zu beachten. Extrapolationen um 5 %-Einheiten über den angegebenen OMDh-Bereich hinaus sind möglich (ausgenommen Rationen), jedoch mit einer etwas größeren Unsicherheit verbunden.

Für **Zucker- und Futterrüben, Getreide** (möglicherweise einschließlich auch für Hafer), großenteils für Erzeugnisse und Nebenerzeugnisse ihrer Verarbeitung sowie eine Reihe von hier nicht näher zu bezeichnenden **Konzentratfuttermitteln**, wenn diese eine OMD > 80 % aufweisen, kann im Allgemeinen von einer nahezu gleich hohen OMD bei Schaf und Pferd ausgegangen werden. Es können jedoch auch deutliche Unterschiede bei bestimmten Futtermitteln auftreten. Für eine Reihe von Futtermitteln verbleibt daher eine Unsicherheit hinsichtlich ihres energetischen Futterwerts, dieser ist dann von einer fachkundigen Person einzuschätzen. Die Ermittlung der OMD oder des ME-Gehalts von **Mischfuttermitteln** ist zum aktuellen Zeitpunkt nur bei Kenntnis der Anteile der verwendeten Einzelfuttermittel möglich.

Mit dem hier beschriebenen *dreistufigen Verfahren* kann der ME-Gehalt von Rationen und Futtermitteln aus der OMD zuverlässig berechnet werden. Gegenüber dem bisherigen Verfahren (GfE 2014) erfolgt eine zutreffendere Einstufung, dies gilt insbesondere für proteinreiche Konzentrate. Zusammen mit der hier aufgezeigten Möglichkeit, die OMD vieler Grobfuttermittel mit variabler Zusammensetzung aus der OMD beim Schaf abzuleiten sowie publizierte, in Verdaulichkeitsuntersuchungen ermittelte OMD-Werte von Futtermitteln mit geringer Variabilität zu nutzen, ist ein Vorgehen aufgezeigt, den ME-Gehalt von Futtermitteln und Rationen zu bestimmen. Außerdem bietet dieses Vorgehen eine hohe Flexibilität und Offenheit, neue wissenschaftliche Erkenntnisse zu berücksichtigen sowie Informationen und Elemente aus anderen Bewertungssystemen zu integrieren.

1. Ausgangssituation

Der Ausschuss für Bedarfsnormen (AfBN) der Gesellschaft für Ernährungsphysiologie (GfE) hat die Umsetzbare Energie (ME, metabolisable energy) als Grundlage für die energetische Bewertung der Futtermittel und die Angabe des Energiebedarfs beim Pferd gewählt (GfE 2014). Die Ermittlung des ME-Gehalts eines Futtermittels oder einer Ration erfolgte hierbei nach einer Gleichung zur Schätzung des Gehalts an verdaulicher Energie (DE, digestible energy), welche die Gehalte der Weender Rohnährstoffe Rohprotein (CP, crude protein), Rohfett (CL, crude lipids), Rohfaser (CF, crude fibre) und N-freie Extraktstoffe (NfE) zur Grundlage hat, und deren Koeffizienten für CP um 8 kJ/g und für CF um 2 kJ/g verringert werden, um die Harnenergieverluste (UE, urinary energy) bzw. CH_4-Energieverluste (CH_4-E) zu berücksichtigen. In den letzten Jahren wurde jedoch zunehmend deutlich, dass die von der GfE (2014) angegebene Schätzgleichung die Unterschiede im energetischen Futterwert von Rationen nicht ausreichend abbilden kann und der für eine Futterbewertung erforderlichen Genauigkeit nicht genügt, wie dies u. a. aus den Arbeiten von Zeyner und Kienzle (2002), Kuchler et al. (2020), Marín et al. (2022) sowie Pankratz (2022) hervorgeht; des Weiteren ist diese Schätzgleichung auf viele Einzelfuttermittel nicht anwendbar. Die Entwicklung eines Verfahrens, das eine zutreffendere Ermittlung der ME ermöglicht, war daher notwendig. Dies veranlasste den AfBN dazu, das beim Wiederkäuer eingeführte *dreistufige Verfahren* zur Bestimmung der ME (GfE 2023) nach tierartspezifischer Anpassung auf die Bewertung von Futtermitteln für Pferde anzuwenden. Dieses Verfahren geht von der Verdaulichkeit der Organischen Masse (OMD, organic matter digestibility) aus, von der die Verdaulichkeit der Energie (ED, energy digestibility) abgeleitet wird, und nimmt eine getrennte Schätzung von UE und CH_4-E vor. Die Prinzipien und Vorzüge des *dreistufigen Verfahrens* sind von der GfE (2023) für Wiederkäuer eingehend begründet und detailliert beschrieben und gelten in entsprechender Weise auch für Pferde.

2. Die Ermittlung der Verdaulichkeit der Energie sowie der Energieabgabe über Harn und Methan nach dem *dreistufigen Verfahren*

2.1 Verdaulichkeit der Energie

Es besteht eine enge Beziehung zwischen der OMD und der ED, sodass sich der Gehalt an DE aus dem Brennwert (GE, gross energy) und der OMD ableiten lässt. Die ED ist um einen konstanten Betrag geringer als die OMD. Diese Differenz beträgt beim Wiederkäuer im Mittel 3,3 und beim Schwein 2,8 %-Einheiten und ist unabhängig vom Futteraufnahmeniveau (FAN) und von der Höhe der OMD (GfE 2023). Die Ableitung eines entsprechenden Werts beim Pferd erfolgte aufgrund folgender Studien:[6] Die beiden von Martin-Rosset und Dulphy (1987) durchgeführten Versuche auf der Basis von Grasheu bzw. Maissilage mit sehr unterschiedlichen Konzentratfutter (KF)-Anteilen und FAN zeigten eine mittlere konstante OMD-ED-Differenz von 4,1 bzw. 2,1 %-Einheiten; die OMD dieser Rationen variierte zwischen 50 und 72 % bzw. 71 und 82 %. Ob ein solcher Unterschied zwischen Grasheu- und Maissilage-basierten Rationen generell auftritt, kann nicht abschließend beurteilt werden. Für die von Martin-Rosset et al. (1990) bei 6 verschiedenen Tiergruppen eingesetzten Rationen mit zwischen 52 und 57 % liegender OMD lag die mittlere Differenz bei 3,6 %-Einheiten. Die Ergebnisse aus der Untersuchung von Zeyner et al. (1992) mit 9 Rationen (55–78 % OMD) ergaben einen Mittelwert der OMD-ED-Differenz von 1,9 %-Einheiten. Bei den von Vermorel et al. (1997a) untersuchten 8 Rationen mit zwischen 51 und 65 % variierender OMD betrug die Differenz 4,3 %-Einheiten und bei den von Vermorel et al. (1997b) untersuchten 14 Rationen zwischen 45 und 70 % OMD 4,5 %-Einheiten. Die bei Zeyner (2002) untersuchten 9 fettarmen, auf Wiesenheu und Mischfutter basierenden Rationen (59–73 % OMD) ergaben eine mittlere Differenz von 3,9 %-Einheiten. Nach Harris et al. (2017) betrug die Differenz von 8 ausschließlich auf Heulage basierenden Rationen (49–75 % OMD) im Mittel 2,4 %-Einheiten. Die 8 von Fehrle (1999) geprüften Rationen (32–79 % OMD) können ebenfalls herangezogen werden, wenn unterstellt wird, dass die Verdaulichkeit der Trockenmasse (DM, dry matter) um 1,6 %-Einheiten geringer ist als die OMD (abgeleitet aus Martin-Rosset et al. 1990, Vermorel et al. 1997a,b, Harris et al. 2017, Särkijärvi et al. 2012, Saastamoinen et al. 2020, 2021), und zeigen eine mittlere OMD-ED-Differenz von 2,6 %-Einheiten. Wenn die gleiche Prämisse für die 8 Rationen (53–80 % OMD) aus der Arbeit von Hipp et al. (2018) vorgenommen wird, beträgt die Differenz 4,2 %-Einheiten. Diese geringen Unterschiede der OMD-ED-Differenzen aus den verschiedenen Arbeiten erlaubt die Bildung eines Mittelwerts dieser Einzeluntersuchungen, der 3,4 %-Einheiten beträgt. Daher gilt für das Pferd:

[1] $$ED\ (\%) = OMD\ (\%) - 3{,}4$$

Die Standardabweichung (SD, standard deviation) der mittleren Differenzen innerhalb der Studien beträgt weniger als 1 %-Einheit und zwischen den Studien 1 %-Einheit, so dass bei Anwendung dieses mittleren Wertes der Fehler maximal 0,2 MJ/kg DM gerechnet werden muss. Dieser relativ niedrige Schätzfehler macht deutlich, dass die ED mit hoher Genauigkeit aus der OMD abgeleitet werden kann. Aus der von Martin-Rosset et al. (1994) angegebenen und im System des INRA (2015) übernommenen linearen Gleichung, welche die Abhängigkeit der ED von der OMD differenziert nach KF und Grobfutter beschreibt, leiten sich mittlere OMD-ED-Differenzen für Grobfutter im Bereich zwischen 45 und 65 % OMD von 3,9 %-Einheiten und für KF zwischen 70 und 85 % OMD von 2,9 %-Einheiten ab, womit eine gute Übereinstimmung mit dem für alle Rationen und Futtermittel hier abgeleiteten Wert von 3,4[7] vorliegt. Eine Abhängigkeit der Differenz von der Höhe der OMD liegt nicht vor. Ein Einfluss des FAN auf diese Differenz wurde nur in einer von drei Untersuchungen beobachtet, die hierzu ausgewertet werden konnten, sodass nicht von einem generellen Effekt auszugehen ist.

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[6]Die Ergebnisse der Literaturauswertung von Sales et al. (2013) konnten nicht berücksichtigt werden, da die Anzahl der Studien, die dem jeweiligen Mittelwert für OMD und ED zugrunde liegen, unterschiedlich sind und die angegebene Gleichung zur Schätzung der ED aus der OMD einen linearen Bias zwischen geschätzten und beobachteten ED-Werten aufweist.

[7]Die Höhe des Unterschieds der OMD-ED-Differenz zwischen KF und Grobfutter ist daher relativ gering und liegt innerhalb der RSD (residual standard deviation) dieser von Martin-Rosset et al. (1994) angegebenen Gleichung als auch der SD von Gleichung [1], weshalb eine entsprechende Differenzierung nicht angezeigt erscheint. Es ist anzunehmen, dass ein großer Teil der von Martin-Rosset et al. (1994) angegebenen Gleichung zugrunde liegenden Daten aus den hier aufgeführten Einzeluntersuchungen stammen. Allerdings weist diese in sich eine Widersprüchlichkeit auf, die darin besteht, dass die OMD-ED-Differenzen mit abnehmender OMD geringer werden, ein Zusammenhang, der sich aus den vorliegenden experimentellen Befunden nicht ableiten lässt. Aus diesem Grund wird diese Gleichung nicht zur Anwendung empfohlen

Die mittlere OMD-ED-Differenz von 3,4 gilt für Rationen mit CL-Konzentrationen im Bereich bis zu 4–5 % der DM. Eine geringere Differenz tritt bei Rationen mit höheren Gehalten hoch verdaulicher Fette ein, wenn die fettfreie OM eine deutlich geringere Verdaulichkeit als CL aufweist; diese Situation dürfte beispielsweise bei Zulage von Pflanzenöl zu einer Ration vorliegen. In solchen Fällen ist es zweckmäßig, eine getrennte Berechnung der Ration ohne Fettzulage und der Fettzulage, für die kein Unterschied zwischen der Verdaulichkeit des CL und der ED angenommen wird, durchzuführen.[8]

2.2 Energieabgabe über den Harn

Die Energieverluste über den Harn variieren im Bereich von 3,8–9,4 % der GE bzw. 7,0–11,9 % der DE (Kienzle und Zeyner 2010) und sind damit höher als beim Wiederkäuer. Die Variation der UE ist überwiegend durch die Höhe der CP-Aufnahme bedingt. Eine Auswertung der Literatur und eigener Daten durch Kuchler et al. (2020) führte zu folgender Gleichung, welche die Abhängigkeit der renalen Energieverluste von der CP-Konzentration des Futters (Bereich 5–24 % der DM) beschreibt:

[2] UE (MJ/kg DM) = 0,33 + 0,0043 CP (g/kg DM) (n = 38; RSD = 0,12; R^2 = 0,81)

und gilt für eine Stoffwechselsituation, in der die mit dem verdaulichen CP in den Stoffwechsel gelangende N-Menge zum überwiegenden Teil nicht retiniert sondern mit dem Harn wieder ausgeschieden wird und kein nennenswerter Körperproteinverlust auftritt. Neuere Daten von Pankratz (2022) fügen sich sehr gut in den Wertebereich der Gleichung [2] ein. Die von Vermorel et al. (1997a,b) angegebenen UE-Werte zeigen eine gute Übereinstimmung mit den nach Gleichung [2] geschätzten Werten. Auch weichen ausschließlich auf Grobfutter basierende Rationen im Bereich von 93 bis 200 g CP/kg DM (Ragnarsson 2009) zwar für einige Rationen, nicht jedoch im Mittel vom geschätzten Wert ab, wobei diese Abweichung jedoch keine Abhängigkeit vom CP-Gehalt zeigte[9]

Das konstante Glied der Gleichung [2] gibt den Anteil der UE wieder, der von der CP-Aufnahme unabhängig ist. Dieser Anteil ist durch die unvermeidlichen N-Verluste des Stoffwechsels bedingt und durch Ausscheidung weiterer energiereicher Verbindungen verursacht. Besonders bedeutsam ist Hippursäure, welche aus der Metabolisierung phenolischer Verbindungen aus Lignin-Kohlenhydrat-Komplexen von Gerüstsubstanzen entsteht. Sollte es in Zukunft möglich sein, die Einflüsse auf die von der CP-Aufnahme unabhängige UE quantitativ zu beschreiben, könnte dies durch Modifikation der Konstanten berücksichtigt werden.

Die Steigung der Gleichung [2] kennzeichnet den Energieverlust pro Gramm zusätzlich aufge-nommenem CP, der nur wenig höher ist als der Wert, welcher zu erwarten ist, wenn Protein vollständig desaminiert wird und der darin enthaltene N in Form von Harnstoff ausgeschieden wird. Daher nehmen die Gesamtverluste an UE pro g CP mit zunehmendem CP-Gehalt der Ration ab, wodurch auch sichergestellt wird, dass CP-reiche Futtermittel, die CP-Gehalte oberhalb des untersuchten Bereichs aufweisen und zur Ergänzung in der Ration eingesetzt werden, nicht mehr unterbewertet werden. Die mit Gleichung [2] für Einzelfuttermittel geschätzten UE-Werte sind additiv und erfüllen damit die Voraussetzung für die Rationsberechnung. Die RSD von Gleichung [2] mit 0,12 MJ/kg DM zeigt, dass die UE zum Zwecke der energetischen Futterbewertung mit ausreichender Genauigkeit allein aus dem CP-Gehalt geschätzt werden kann. Es ist noch anzumerken, dass auch für Futtermittel, bei denen keine direkten Harnenergieverluste zu erwarten sind, wie z. B. Pflanzenöle oder reine Stärke, ebenfalls der Energieabzug in Höhe der Konstanten von 0,33 MJ/kg vorgenommen wird, da Gleichung [2] aus Rationen, die Grobfutter enthalten, abgeleitet ist; die hier – möglicherweise auch für phenolarme Futtermittel – auftretende Unzulänglichkeit ist jedoch zu vernachlässigen.

[8] Es ist darauf hinzuweisen, dass der für reine Futterfette in Tabellen angegebene OMD-Wert häufig mit der Verdaulichkeit der CL-Fraktion gleichgesetzt wird, was jedoch den endogenen Verlust ignoriert; aufgrund des endogenen Verlustes ist die OMD geringer als die CL-Verdaulichkeit. Liegt ein Wert für die CL-Verdaulichkeit von reinen Futterfetten vor, sollte dieser als Näherungswert für ED herangezogen werden, da diese beiden Werte einen geringeren Unterschied aufweisen.

[9] Daten bei Vermorel et al. (1997a,b) und Ragnarsson (2009) sind Bestandteil des Datensatzes von Pankratz (2022).

2.3 Energieabgabe über Methan

Die Energieverluste in Form von Methan variieren nach einer Literaturauswertung durch Kienzle und Zeyner (2010) zwischen 1,9 und 4,2 % der GE bzw. 3,4 und 6,9 % der DE und sind somit niedriger als die UE. Die Methanbildung ist beim Pferd deutlich geringer als beim Wiederkäuer, da ein erheblicher Anteil der Nährstoffe im Dünndarm verdaut wird, die der Fermentation nicht unterliegen, und infolge der im Dickdarm umfangreich stattfindenden reduktiven Acetogenese pro Gramm fermentierte Substanz weniger Methan entsteht als in den Vormägen von Wiederkäuern. Die Energieverluste über eine Wasserstoffabgabe (H_2-E) sind gegenüber der CH_4-E gering und liegen im Bereich von 1 bis 7 % der CH_4-E (abgeleitet aus Angaben bei Zentek et al. 1992, Zeyner 2002 und Mößeler et al. 2005). Es ist hierbei unterstellt, dass das gemessene Verhältnis beider Gaskonzentrationen im Exhalat das Verhältnis ihrer gesamten mengenmäßigen Abgabe widerspiegelt. Die Daten bei Mößeler et al. (2005) zeigen, dass die bei bestimmten Rationen auftretende erhöhte H_2-Abgabe mit einer Reduktion der CH_4-Abgabe verbunden ist, wobei jedoch die Reduktion der CH_4-E stärker ausfällt als die Zunahme der H_2-E. Der Verlust an CH_4-E + H_2-E bei Rationen mit geringer H_2-Bildung war deswegen höher als bei hoher H_2-Bildung. Bei den beiden anderen angeführten Untersuchungen konnte weder ein solcher noch ein positiver Zusammenhang beobachtet werden. Die H_2-Bildung hängt überwiegend von der Kohlenhydratquelle [Cellulose + Hemicellulosen (Grünmehl) und Pektine (Trockenschnitzel) < Stärke (Hafer) < Inulin (Topinambur)] ab und scheint von der CH_4-Bildung unabhängig zu sein. Da eine zuverlässige Schätzung der H_2-Abgabe zurzeit nicht möglich ist und darüber hinaus der sehr geringe Anteil der H_2-E für die energetische Futterbewertung nicht relevant ist, wird im *dreistufigen Verfahren* auf eine Berücksichtigung der H_2-E verzichtet.

Die CH_4-Bildung zeigt eine Abhängigkeit vom Gehalt an Faserfraktionen bzw. Nicht-Stärke-Polysacchariden. Eine Fütterungssituation, bei der größere Mengen an Stärke in den Dickdarm gelangen, wird hier nicht betrachtet, da eine solche als nicht physiologisch anzusehen ist und durch eine adäquate Rationsgestaltung vermieden werden sollte (GfE 2014). Aufgrund erhöhter Fermentationsverluste wäre sie nicht geeignet, das Energielieferungspotential eines Futtermittels zu beschreiben. Eine umfassende Literaturauswertung (A. Zeyner, C. Böttger, A. Susenbeth, persönliche Mitteilung)[10] ergab eine mittlere CH_4-E-Bildung von 0,49 MJ/kg DM (SD = 0,11); diese liegt innerhalb der Spanne von 0,35 bis 0,60 MJ/kg DM in der Literatur angegebener Mittelwerte (Kienzle und Zeyner 2010). Die CH_4-E/g CF betrug 1,99 kJ (SD = 0,38) und war von der CF-Konzentration nicht beeinflusst. Der Bezug zum Organischen Rest [OR = OM − CP − CL − ST (Stärke) − ZU (Zucker)] wies ebenfalls keine Abhängigkeit von der Konzentration an OR auf. Mit dem berechneten OR sind auf einfache Weise weitgehend alle Nicht-Stärke-Polysaccharide erfasst. Die Methanenergieverluste (CH_4-E) betragen somit im Mittel

[3] $CH_4\text{-E} = 0{,}83$ kJ/g OR (n = 60; SD = 0,16; SD% = 19; Spanne 486–736 OR, g/kg DM)

Ein Einfluss der Futteraufnahmehöhe auf die CH_4-Abgabe pro g CF bzw. OR lag für den Bereich von 4,7 – 8,0 kg DM-Aufnahme/Tag nicht vor. Wird mit Hilfe dieses Werts die CH_4-E für eine Ration geschätzt, beträgt der dabei auftretende Fehler (SD) ca. 0,10 kJ/g DM. Dieser ist geringer als derjenige, der bei der Ableitung der ED aus der OMD auftritt, und etwa gleich groß wie bei der UE-Schätzung. Die Kenntnis der CF-Konzentration eines Futtermittels ist somit zur Schätzung der CH_4-Abgabe nicht erforderlich.

[10]Die Datenbasis bildeten die Ergebnisse der Untersuchungen von Fingerling aus den Jahren 1931–1939. Dabei handelt es sich um insgesamt 60 geprüfte Rationen. Die Grundrationen bestanden entweder aus Wiesenheu, Haferkörnern, Leinsamenmehl und Melasse oder nur Wiesenheu. Folgende Futtermittel wurden im Differenzversuch geprüft: Stroh unterschiedlicher botanischer Herkunft, Strohstoff, Wiesenheu unterschiedlicher Schnittzeitpunkte, Luzerne- und Kleeheu, Hafer, Maisschrot, Roggenkleie, Weizen- und Reiskleber, Futterrüben, Zucker, Kartoffelflocken, Kartoffelstärke, Pferdebohnen, Biertreber, Kakaoschalen, Erdnussöl. Die Gehalte an ST und ZU wurden aufgrund der verwendeten Futtermittel und deren Anteile in der Ration aus entsprechenden Angaben in Futterwerttabellen abgeleitet. Die mittleren Gehalte, deren SD und Spanne betrugen pro kg DM für CP 127, 19,0, 102–237, für CL 53, 12,4, 23–107, für CF 250, 41,2, 173–361, für ST + ZU 156, 58,7, 66–299, und für OR 596, 62,8, 486–736.

2.4 Einflüsse des Futteraufnahmeniveaus, der Rasse und des physiologischen Status auf die Verdaulichkeit der Organischen Masse

Zum Einfluss des Futteraufnahmeniveaus (FAN) auf die OMD liegen für das Pferd nur wenige Untersuchungen vor, die vor allem auf die Arbeitsgruppe um Martin-Rosset zurückgehen (Martin-Rosset et al. 1987, Martin-Rosset und Dulphy 1987, Martin-Rosset et al. 1990). Das Futteraufnahmeniveau 1 (FAN1) wurde von der GfE (2023) definiert als 50 g DM/Einheit metabolischer Körpergröße ($kg^{0,75}$)[11] und wird hier übernommen. Die Einschätzung des Effekts auf die OMD im Energiebewertungssystem nach Vermorel et al. (1997c) und des INRA (2015) basiert auf den Ergebnissen dieser Untersuchungen. Der AfBN übernimmt im Wesentlichen die daraus für das System des INRA (2015) abgeleiteten Schlussfolgerungen, in dem allerdings der mögliche Einfluss des FAN auf die Methanbildung kein Gegenstand ist. Es wird davon ausgegangen, dass bei einer Erhöhung des FAN von 1 auf 2–2,5 (d. h. auf 100–125 g DM/$kg^{0,75}$ KM) keine Reduktion der OMD auftritt. Ein FAN in dieser Größenordnung wird in der Regel auch in der Laktation oder bei Arbeit nicht wesentlich überschritten (GfE 2014). Die Ergebnisse von Ragnarsson (2009), wonach eine geringere OMD und ED bei höherem FAN auftrat, sind hier nicht zu berücksichtigen, da das niedrige FAN in dieser Untersuchung erheblich unter 1 lag. Ferner wird angenommen, dass sich die OMD zwischen leichten und schwereren Rassen nicht unterscheidet, Ponys jedoch gegenüber leichten Rassen eine geringfügige, um 2 %-Einheiten höhere Verdaulichkeit aufweisen, wobei dieser Wert nach Hoffmann et al. (1987) nicht als konstant anzusehen ist. Die Annahme einer etwas höheren OMD beim Pony wird beibehalten, obwohl in der Studie von Potter et al. (2022) dieser Effekt nicht auftrat, was möglicherweise dadurch bedingt war, dass die Kotsammlung nur über 1 Tag erfolgte, die Anzahl der Tiere gering war und die Körpermasseunterschiede zwischen den Rassen nicht sehr groß waren. Auch wird davon ausgegangen, dass Unterschiede in der Verdaulichkeit zwischen abgesetzten jungen und ausgewachsenen Tieren nicht auftreten (Martin-Rosset und Dulphy 1987, Harris et al. 2017); des Weiteren, dass die Verdaulichkeit zwischen laktierenden und nicht-laktierenden Stuten sich trotz deutlich unterschiedlicher Futteraufnahme nicht unterscheidet, bei Stuten im 8.–11. Trächtigkeitsmonat jedoch reduziert ist. Ein Einfluss einer leichten bis moderaten Arbeit bei Rationen mit mindestens 40 % Grobfutter und einem FAN von 2 bis 2,5 wird nicht angenommen.

Martin-Rosset und Dulphy (1987) konnten anhand von Rationen mit Grasheu und Maissilage zeigen, dass auch bei hohen KF-Anteilen (bis 60 bzw. 90 % der Ration) die Additivität der Verdaulichkeitswerte gegeben war und auch keine Interaktion mit dem FAN auftrat; die Verdaulichkeit des Grobfutters war von dessen Anteil in der Ration unabhängig, im Unterschied zu Beobachtungen in gleichzeitig durchgeführten Verdaulichkeitsversuchen am Schaf.

Zusammenfassend kann festgehalten werden, dass die OMD in der Bewertung von Futtermitteln für Pferde eine Größe ist, bei der eine Berücksichtigung des FAN, der Rationszusammensetzung und des physiologischen Zustandes (mit Ausnahme der hochtragenden Stute) gemäß derzeitigem Kenntnisstand nicht erforderlich ist. Untersuchungen und Überlegungen zu möglichen Einflüssen der genannten Faktoren auf die Methanbildung wurden in der Literatur nicht gefunden. Aufgrund der relativ geringen Methanbildung wären solche Einflüsse von geringer quantitativer Bedeutung.

[11]Das FAN1 ist nicht mit dem Ernährungsniveau 1 gleichzusetzen. Bei einer OMD der Ration von 65 % entspricht dies jedoch einer energetischen Versorgung im Bereich des Erhaltungsbedarfs, wenn hierfür 0,50 MJ ME/kg0,75 KM unterstellt werden. Dies kommt mit dem Wert von 32 g verdaulicher OM/kg0,75 KM etwa überein, bei dem nach Jarrige und Martin-Rosset (1984) (zit. bei Martin-Rosset et al. 1990) der energetische Erhaltungsbedarf gedeckt ist. Verbreitet ist auch die Definition des FAN als DM-Aufnahme in % der KM. Bei einer KM von 600 bis 650 kg führen beide Bezüge etwa zum gleichen Wert (FAN1 = 6,1 bzw. 6,4 kg DM).

3. Ermittlung der Bruttoenergie und der Verdaulichkeit der Organischen Masse

3.1 Bestimmung des Brennwerts

Die Brennwert des Futtermittels stellt dessen Bruttoenergie (GE) dar und wird mittels Kalorimetrie bestimmt (DIN, 2003). Wenn eine kalorimetrische Bestimmung nicht möglich ist, kann eine Berechnung mit folgender Gleichung vorgenommen werden (Hoffmann et al. 1993, Beyer et al. 2003):

[4] GE (kJ/kg DM) = 23,6 CP + 39,8 CL + 17,3 ST + 16,0 ZU + 18,9 OR (jeweils g/kg DM)

wobei CP, CL, ST, ZU und OR Rohprotein, Rohfett, Stärke, Zucker und Organischer Rest bedeuten.[12] Gleichung [4] liefert zuverlässige Ergebnisse für Rationen und die meisten Futtermittel. Sie birgt zwar für einige auch Ungenauigkeiten (siehe hierzu GfE 2023), die jedoch je nach Anwendung bestimmter Verfahren mit größeren Fehlerquellen, z. B. der Bestimmung der OMD beim Pferd (OMDh) aus OMD beim Schaf (OMDs; siehe Abschnitt 3.2.3), für die meisten Futtermittel als nachrangig anzusehen sind. Für die von der GfE (1995) angegebene Schätzgleichung (übernommen von Hoffman et al. 1971), die auf den Gehalten an CP, CL, CF und N-freien Extraktstoffen beruht, kann nach der Untersuchung von Pankratz (2022) für Gehalte, die sich im Bereich von 18,0–18,5 MJ/kg DM bewegen, zwar eine im Mittel gute Übereinstimmung mit den kalorimetrisch bestimmten Brennwerten erwartet werden, sie weist jedoch eine erhebliche Streuung und teilweise deutliche Abweichungen bei niedrigeren und höheren Brennwerten auf, und wird daher nicht zur Anwendung empfohlen.

3.2 Ermittlung der OMD

3.2.1 Bestimmung der OMD im Verdaulichkeitsversuch

Der Aufwand zur Bestimmung der OMD bzw. ED im Verdaulichkeitsversuch ist sehr hoch und die Anzahl publizierter, *in-vivo* bestimmter OMD-Werte begrenzt. Diese Daten sind für die Rationsplanung in der Praxis insbesondere dann hilfreich, wenn es sich um Futtermittel handelt, die nur eine geringe Variation in den Gehalten an Inhaltsstoffen und deren Verdaulichkeiten aufweisen und eindeutig einer gut definierten Futtermittelgruppe zuzuordnen sind. Wie in den folgenden Abschnitten ausgeführt, sind die bei der Schätzung der OMD über *in-vitro*-Verfahren oder aus der OMD beim Schaf auftretenden Fehler und die damit verbundene Unsicherheit für einen beachtlichen Teil der Futtermittel teilweise erheblich; diese unterstreichen die Bedeutung und Notwendigkeit von Verdaulichkeitsversuchen beim Pferd, deren Genauigkeit und Zuverlässigkeit nicht durch anderen Methoden zu erreichen ist. Verdaulichkeitsmessungen am Tier als Referenzmethode sind daher insbesondere als Grundlage für die Entwicklung und Überprüfung von Alternativen zum Verdaulichkeitsversuch unumgänglich.

3.2.2 Schätzung der OMD mit *in-vitro*-Methoden

In-vitro-Methoden haben im Bereich der Wiederkäuerernährung eine große Bedeutung erlangt. Mit ihnen ist es möglich, zuverlässig und mit ausreichender Genauigkeit die OMD zu schätzen (GfE 2023). Je nach Futtermittelgruppe ist dabei mit einer RSD von 2–5 %-Einheiten zu rechnen. Für das Pferd wurde in einer Reihe von Studien die Eignung von *in-vitro*-Methoden überprüft und entsprechende Schätzgleichungen wurden publiziert. Dem AfBN dienten als Grundlage für die Einschätzung ihrer Eignung in der praktischen Futterbewertung die Arbeiten von Smolders et al. (1990), Macheboeuf et al. (1998a/b), Zeyner und Dittrich (2005), Martin-Rosset et al. (2012) und INRA (2015). Bei Verwendung dieser Arbeiten ist zu beachten, dass ähnlich bezeichnete *in-vitro*-Methoden nicht immer identisch sind, weshalb bei der Anwendung einer Schätzgleichung die hierbei zugrunde liegenden Methodenschritte genau zu beachten sind.

Der AfBN schließt sich der Auffassung von Martin-Rosset et al. (2012) an, dass die Eignung von *in-vitro*-Methoden beim Pferd grundsätzlich gegeben ist. Die von Martin-Rosset et al. (2012) entwickelte Schätzgleichung für Grobfutter (RSD = 2,0) und die bei INRA (2015) angegebene Gleichung für KF („processed ingredients“; RSD = 5,6), die einen enzymatischen Abbau der OM *in-vitro* zur Grundlage haben, sowie die Ergebnisse von Macheboeuf et al. (1998a/b) für Grünlandaufwuchs und Luzerne, die auf der 24-h-*in-vitro*-Gasbildung mit Caecuminhalt (RSD = 2,7 bzw. 2,2) oder mit Kot von Pferden (RSD = 2,8 bzw. 2,5) als Inokula basieren, können nach Ansicht des AfBN geeignete Verfahren für die praktische Anwendung darstellen, wenn zuvor eine Standardisierung, Validierung und Überprüfung in

[12]Die verschiedenen Methoden zur Bestimmung von ST und ZU führen zu teilweise nicht unerheblichen Gehaltsunterschieden, die damit auch entsprechende Unterschiede im Gehalt an OR zur Folge haben. Die nach dieser Gleichung berechneten GE-Gehalte sind jedoch dadurch nur in relativ geringem Umfang betroffen, da sich die Koeffizienten für ST, ZU und OR nicht sehr stark unterscheiden und die Unterschiede im analysierten Gehalt sich daher nur entsprechend des Unterschieds ihrer Koeffizienten auswirken. Für bestimmte Einzelfuttermittel sind die Hinweise bei den jeweiligen Methoden des VDLUFA (2012) zu beachten.

Ringuntersuchungen durchgeführt wurde.

In-vitro-Methoden bieten demnach zukünftig ein großes Potential für eine routinemäßige und zuverlässige Bestimmung der OMD. Eine Möglichkeit, den sehr hohen Aufwand von Verdaulichkeitsversuchen zur Erstellung von Referenzwerten für eine ausreichend große Serie von Futtermitteln zu umgehen, der zur Entwicklung und Überprüfung einer *in-vitro*-Methode erforderlich ist, könnte darin bestehen, nur einige wenige Grob- und Konzentratfuttermittel sowie Mischfuttermittel im Verdaulichkeitsversuch und mit einem etablierten *in-vitro*-Verfahren zu prüfen, um diese als Standards zu verwenden. Die gemessene Gasbildung bzw. die enzymlösliche OM dieser Standardfuttermittel ermöglichen dann die Berechnung eines Korrekturfaktors. Ein nicht unerheblicher Anteil der Reststreuung von Regressionsgleichungen zur Schätzung der OMDh dürfte jedoch auch auf die Streuung der *in-vivo* bestimmten Werte zurückzuführen sein, da innerhalb einer Testration die OMD-Werte beim Pferd und Pony individuell deutlich stärker variieren als beim Schaf (Hoffmann et al. 1987), was eine ent-sprechende Tierzahl und Kotsammeldauer erforderlich macht.[13]

3.2.3 Ableitung der OMD beim Pferd aus der OMD beim Schaf

Die OMD beim Pferd (OMDh) steht in einer Beziehung zur OMD beim Schaf (OMDs), da beide Spezies aufgrund ihres Verdauungssystems in der Lage sind, große Mengen Nicht-Stärke-Polysaccharide durch Fermentation abzubauen. Hierauf beruht die Möglichkeit, die OMDh aus der OMDs abzuleiten, was den entscheidenden Vorteil mit sich bringt, dass umfangreiche Daten aus Verdaulichkeitsuntersuchungen an Schafen herangezogen werden können, wohingegen Ergebnisse aus Verdaulichkeitsversuchen beim Pferd nur in begrenztem Umfang und für die Untersuchungspraxis geprüfte Alternativmethoden noch nicht zur Verfügung stehen. Generell ist die OMDh bei Grobfutter und gemischten Rationen geringer ist als die OMDs. Die Differenz zwischen OMDh und OMDs ist bei faserreichem Grobfutter größer, nimmt mit zunehmender OMD ab, und tritt in der Regel bei höherverdaulichen Futtermitteln oder Rationen nicht auf. So konnten Särkijärvi et al. (2012) zeigen, dass bei Grassilagen die Verdaulichkeit der Neutral-Detergenzien-Faser (NDF, neutral detergent fibre)-freien OM nicht verschieden, jedoch die NDF-Verdaulichkeit beim Pferd deutlich geringer ist als beim Schaf und die Differenz mit zunehmender NDF-Verdaulichkeit kleiner wird. Schon Langworthy (1903), Fingerling (1940, zit. bei Axelsson 1941) und Axelsson (1941) hatten darauf hingewiesen, dass der Unterschied in der Verdaulichkeit bei CF gegenüber anderen Rohnährstoffen am stärksten, jedoch nicht einheitlich ausgeprägt ist. Damit hängt die OMDs-OMDh-Differenz im Wesentlichen von der Konzentration und der Verdaulichkeit der Faserfraktionen ab. Jedoch kann diese Differenz für bestimmte Futtermittelgruppen einen Wert aufweisen, der sich nicht auf den Fasergehalt oder die Verdaulichkeit zurückführen lässt. Es müssen also noch andere Faktoren, beispielsweise die Passagerate, eine Rolle spielen, so dass einer Generalisierung der Beziehung zwischen OMDh und OMDs Grenzen gesetzt sind. Der nachfolgende Vorschlag zur Schätzung der OMDh aus der OMDs beruht im Wesentlichen auf den Arbeiten von Martin-Rosset et al. (1984), Schmolders et al. (1990) und Särkijärvi et al. (2012) sowie einer Gleichung von Zeyner et al. (1994).[14] Neben der Schätzgleichung für Rationen (Zeyner et al. 1994) finden sich in den anderen Arbeiten differenzierte Schätzgleichungen für bestimmte Futtermittelgruppen, die jedoch nicht immer so streng definiert sind, dass ein bestimmtes Futtermittel sich eindeutig zuordnen ließe, wodurch ein Vergleich erschwert und eine Übereinstimmung oder Abweichung zwischen Studien nicht sicher auszumachen ist. Die verschiedenen linearen Regressionsgleichungen nach der Form OMDh (%) = b_0 + b_1 OMDs (%) wurden hinsichtlich des zugrundeliegenden Datenumfangs und der Plausibilität ihrer Funktionswerte geprüft und bewertet. Als nicht plausibel wurden Gleichungen eingestuft, die innerhalb einer Futtermittelgruppe eine sich vergrößernde Differenz zwischen OMDh und OMDs mit zunehmender OMD aufweisen, und solche, die im Bereich mittlerer Verdaulichkeit eine deutlich höhere OMD beim Pferd als beim Schaf zeigen.

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[13]Auf eine geringe Wiederholbarkeit von OMD-Werten in ihren Versuchen wiesen Goachet et al. (2012) hin, möglicherweise bedingt durch zu kurze Kotsammelperioden. Hinweise zur Durchführung von Verdaulichkeitsuntersuchen sind bei Zeyner (2005) zu finden, wobei in Ergänzung eine Sammelperiode von mindestens 5 Tagen einzuhalten ist. Im Falle des Einsatzes eines Markers ist dessen Wiederfindung und deren SD zu bestimmen. – Den physiologischen Ursachen für die relativ große Streuung der Verdaulichkeit sollte nachgegangen werden, um sie möglicherweise im Verdaulichkeitsversuch berücksichtigen zu können. – Auf die Notwendigkeit einer gewissen körperlichen Bewegung der Tiere in Verdaulichkeitsversuchen wurde schon früh hingewiesen (Langworthy 1903).

[14]In der angegebenen Gleichung ist augenscheinlich die Bezeichnung der abhängigen und unabhängigen Variablen vertauscht, was hier berücksichtigt wurde.

Vier dieser Gleichungen,[15] die diese Prüfkriterien erfüllen, zeigen einen gut übereinstimmenden b_1-Wert, obwohl die verschiedenen Futtermittelgruppen unterschiedlich große Differenzen zwischen OMDh und OMDs aufwiesen, die vor allem über den b_0-Wert abgebildet werden. Daher ist es angebracht, einen mittleren b1-Wert zu berechnen und als allgemein zutreffend anzunehmen. Der b_1-Wert gibt an, um wieviel %-Einheiten die Differenz zwischen OMDs und OMDh sich verringert, wenn die OMDs um 1 %-Einheit zunimmt. In dieser Weise kann eine gewisse Allgemeingültigkeit erreicht werden, wenn über entsprechende b_0-Werte (hier als K-Wert angegeben) der Unterschiedlichkeit verschiedener Futtermittel Rechnung getragen wird. Die OMDh kann für einige Grobfuttergruppen und Rationen näherungsweise aus der OMDs wie folgt abgeleitet werden:

[5] $$OMDh\ (\%) = -K + 1{,}15\ OMDs\ (\%)$$

mit folgenden K-Werten für einzelne Futtermittelgruppen:

K	Futtermittelgruppe	abgeleitet aus dem OMDh-Bereich (%)
11,5	Luzerne[16]	51–62
14,5	Grünlandaufwuchs[17]	36–76
17,5	Grasbestand aus Deutschem Weidelgras *(Lolium perenne)*[18]	46–65
22	Grasbestand[19] aus Wiesenschwingel *(Festuca pratensis)* oder Rohrschwingel *(Festuca arundinacea)*	46–67
10,5	Gemischte Rationen[20]	55–70

Die Differenz zwischen OMDs und OMDh liegt für Rationen innerhalb der untersuchten Bereiche demnach zwischen 2,0 und 0. Bei ca. 70 % OMD weisen Pferd und Schaf etwa den gleichen Wert auf, was durch die Ergebnisse der Untersuchungen von Martin-Rosset und Dulphy (1987) sowie von Hoffmann et al. (1987) mit überwiegend aus Grasheu und höheren Anteilen an KF bestehenden Rationen gestützt wird. Ein sehr geringer Unterschied bei OMD >70 % ergibt sich auch aus den beiden von Axelsson (1941) angegebenen Gleichungen.[21] Eine Anwendung auf Rationen >70 % OMD ist nicht zulässig.[22] Für Luzerne liegt die Differenz zwischen 3 und 2, für Grünlandaufwüchse zwischen 8 und 3, für Grasbestände aus Weidelgras zwischen 9 und 7, und solche aus Wiesen- oder Rohrschwingel zwischen

[15]Es handelt sich hierbei um Gleichungen für folgende Futtermittelgruppen: Grünlandaufwuchs und Luzerne (Martin-Rosset et al. 1984), Grasbestand aus Deutschem Weidelgras (Smolders et al. 1990), Grasbestand bestehend überwiegend aus Wiesen- und Rohrschwingel (Särkijärvi et al. 2012).

[16]In der hier zugrunde liegenden Publikation (Martin-Rosset et al. 1984) wird die umfassendere Bezeichnung Leguminosen gewählt, obwohl der überwiegende Anteil der Daten an Luzerne erhoben wurde und nur 20 % andere Grobfutterleguminosen ausmachten. Es erfolgt hier die Einschränkung auf Luzerne, auch aufgrund der guten Übereinstimmung mit Werten der DLG-Futterwerttabellen (Universität Hohenheim – Dokumentationsstelle 1995 und 1997; siehe Abschnitt 3.3).

[17]In der hier zugrunde liegenden Publikation (Martin-Rosset et al. 1984) wird die umfassendere Bezeichnung Grünlandaufwuchs und Grasbestand verwendet, obwohl nur ca. 15 % der Daten an Grasbeständen und 10 % an Strohrationen erhoben wurden. Die Untersuchungen wurden vor dem Jahr 1984 in Frankreich durchgeführt. Der hier angegeben K-Wert ist nur für ähnliche von INRA untersuchte Grünlandaufwüchse sicher anzuwenden. Ein erheblicher Umfang von Literaturdaten für Grünlandaufwüchse wurde durch die Autoren von der Auswertung ausgeschlossen mit der Begründung, dass diese deutlich größere OMDs-OMDh-Differenzen aufweisen und aus Untersuchungen stammen, die lange zurückliegen und an arbeitenden Pferden vorgenommen wurden; diese Daten führen zu einem K-Wert von ca. 21,5.

[18]Der K-Wert gilt nicht für künstlich getrocknetes Gras (Smolders et al. 1990).

[19]Eine der 6 hier untersuchten Rationen bestand aus Wiesenlieschgras (Phleum pratense). – Die Untersuchung mit Wiesenlieschgras von Udén und Van Soest (1982) wurde nicht berücksichtigt, da nur zwei Tiere verwendet wurden und deren Verdaulichkeit sich deutlich unterschied.

[20]Diese Gruppe weist gegenüber den anderen eine größere Heterogenität in der Zusammensetzung und dadurch auch eine größere Reststreuung auf. Die Experimente von Hoffman et al. (1987) zeigen, dass bei hoher OMD die OMDh auch über der OMDs liegen kann; aus deren Ergebnissen kann ebenfalls ein K-Wert für Rationen in einer ähnlichen Größenordnung wie der oben angegebenen abgeleitet werden.

[21]Axelsson (1941) gibt jeweils für Pferde und Rinder eine lineare Gleichung an, die den Einfluss der CF-Konzentration auf die OMD beschreiben.

[22]Es liegen zwar Beobachtungen vor, dass bei Rationen mit geringem Grobfutteranteil die OMDh auch über der OMDs lag (Hoffman et al. 1987), welche jedoch nicht verallgemeinert werden können. Hingegen dürfte bei Grobfutter eine Extrapolation auf höhere OMD-Werte um 5 %-Einheiten noch zu zuverlässigen Werten führen.

13 und 10 %-Einheiten für die spezifizierten OMDh-Bereiche. Die Genauigkeit der Schätzung für Luzerne, Grünlandaufwüchse und Grasbestände, ausgedrückt als RSD, kann in Anlehnung an die Originalgleichungen im Bereich von 1 bis 2,5 angenommen werden, diejenige für Rationen mit 5,5.[23] Die hier vorgelegte Gleichung [5][24] gibt die durch die oben genannten vier Gleichungen der Literatur beschriebenen Zusammenhänge zwischen OMDs und OMDh übereinstimmend wieder.

Bei Anwendung der für Gleichung [5] angegebenen K-Werte ist zu beachten, dass diese zunächst nur für das den Untersuchungen zugrunde liegende Material Gültigkeit besitzen, was angesichts der Heterogenität der Grünlandaufwüchse, Grasbestände und Rationen zu beachten ist, wodurch eine sichere Zuordnung des einzuschätzenden Futtermittels nicht immer möglich ist. Es kann auch nicht ausgeschlossen werden, dass die für Grünlandaufwüchse und Grasbestände vorhandenen Unterschiede nicht nur durch die botanische Zusammensetzung, sondern auch durch Standortfaktoren und die Versuchsmethodik verursacht wurden. So ist der K-Wert für Grünlandaufwuchs und Luzerne aus Untersuchungen an französischen, [25] für Grasbestände aus Weidelgras an niederländischen, und derjenige für die anderen Grasbestände an finnischen Standorten abgeleitet. Die Konservierungsart (Heu, Silage) hat nach Müller (2012) keinen Einfluss auf die OMD; außerdem war die OMDs-OMDh-Differenz zwischen Heu und frischem Grünfutter nicht verschieden (Martin-Rosset et al. 1984; Smolders et al. 1990). Auch hatte nach Todd et al. (1995) die mechanische Aufbereitung von getrockneter Luzerne (lange Partikel, gehäckselt, gepresst, pelletiert) keinen Effekt auf die DM-Verdaulichkeit (DMD, dry matter digestibility) und die ED. Daher kann nach heutiger Kenntnis der mit Gleichung [5] beschriebene Zusammenhang als unabhängig von einer Konservierung und der Partikelgröße betrachtet werden.

Bei anderen als den zuvor genannten Futtermittelgruppen, möglicherweise auch bei einzelnen Futtermitteln, treten keine OMD-abhängigen, sondern mittlere Differenzen auf, die jedoch sehr uneinheitlich ausfallen. So betrug nach Schmolders et al. (1990) die mittlere Differenz zwischen OMDs und OMDh für einige Erzeugnisse und Nebenerzeugnisse der Müllerei 2 %-Einheiten (SD = 4), für Getreide und Körnerleguminosen 4 %-Einheiten (SD = 6); für Mischfutter war die OMDh um 3,5 %-Einheiten (SD < 2) höher als OMDs.[26] Die SD-Werte zeigen die relativ große Uneinheitlichkeit dieser Differenz; auch ist die Datenbasis mit jeweils 3 Futtermitteln gering, so dass diese Werte sowie die Unterschiede zwischen diesen Futtermittelgruppen nicht zu verallgemeinern sind.

Mit Gleichung [5] für die oben genannten Grobfuttermittel und gesicherten Werten von OMDs-OMDh-Differenzen anderer Grobfutter und einiger KF (siehe Abschnitt 3.3) ist ein Orientierungsrahmen zur Schätzung der OMDh aus der OMDs gegeben. Für andere Futtermittel ist mit einem teilweise höheren Fehler und einer größeren Unsicherheit zu rechnen, die sich noch erhöhen dürften, wenn Einzelfuttermittel nicht präzise zugeordnet werden können oder eine Extrapolation erfolgen muss, die über den zugrundeliegenden untersuchten Bereich hinausgeht. Die hier vorgenommene Differenzierung nach Futtermittelgruppen erlaubt zwar für einige Grobfuttertypen eine für die Praxis ausreichend zuverlässige Einschätzung, für andere Grobfutter (z. B. Grobfutterleguminosen) ist eine entsprechende Datengrundlage jedoch nicht vorhanden. Eine Verbesserung der jeweiligen Schätzgenauigkeit kann erreicht werden, wenn zusätzlich zur OMDs der NDF-Gehalt als Variable berücksichtigt wird (Smolders et al. 1990). Die für eine allgemeine Anwendung entsprechender publizierter Gleichungen erforderliche gesicherte Grundlage erscheint dem AfBN jedoch nicht gegeben zu sein.

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[23]Bei der Beurteilung der SD, die bei der Schätzung von OMDh aus OMDs auftritt, ist zu beachten, dass die Bestimmung der jeweiligen OMD ebenfalls mit einem nicht unerheblichen Fehler behaftet sein kann, so dass davon auszugehen ist, dass die tatsächliche mittlere Abweichung zwischen OMDh und OMDs geringer und die Schätzgenauigkeit höher ist.

[24]Ein prinzipiell ähnliches Modell wurde schon von Smolders et al. (1990) für Gras verschiedener Konservierungsarten vorgeschlagen.

[25]Hier ist eine Präzisierung erforderlich: auf die in der Literatur häufig Bezug genommene, von Martin-Rosset et al. (1984) angegebene Gleichung für Grünlandaufwüchse beruht ausschließlich auf Daten, die vom INRA erarbeitet wurden, wogegen bei derjenigen für Grobfutterleguminosen auch Daten von Luzerne und Rotklee aus der Literatur herangezogen wurden. Nach Smolders et al. (1990) könnte es sein, dass die geringere OMDs-OMDh-Differenz für diese Grünlandaufwüchse gegenüber den eigenen Ergebnissen mit Deutschem Weidelgras auf deren höheres FAN der Schafe zurückzuführen ist. Träfe dies zu, würde sich ein entsprechend angepasster K-Wert nur wenig von dem für den Grasbestand aus Deutschem Weidelgras unterscheiden.

[26]Eine Erklärung konnte vonseiten der Autoren nicht angegeben werden; Mahlen und Pelletieren als Ursache werden ausgeschlossen, da diese nach mehreren Quellen die Verdaulichkeit nicht beeinflussen.

Bezüglich der Genauigkeit der OMDh-Schätzung aus der OMDs sei ergänzend erwähnt, dass Schätzgleichungen zur Berechnung der OMD beim Wiederkäuer unter Zuhilfenahme von *in-vitro*-Daten ebenfalls RSD-Werte zwischen 2 und 3, einige auch bis 5 %-Einheiten aufweisen (GfE 2023). Ein Fehler in derselben Größenordnung ist für *in-vitro*-Verfahren beim Pferd zu erwarten (Smolders et al. 1990), wodurch der hier aufgezeigte Weg, die OMDh aus der OMDs abzuleiten, an relativer Vorzüglichkeit gewinnt, weil ein umfangreiches Datenmaterial für Wiederkäuer bereits vorliegt und *in-vitro*-Methoden etabliert sind. Der Fehler der Schätzung der OMDh aus der OMDs, die wiederum über ein *in-vitro*-Verfahren bestimmt wird, ist durch diesen weiteren Schritt allerdings entsprechend erhöht.[27] Es ist noch auf den Umstand hinzuweisen, dass im Gegensatz zum Schaf das FAN beim Pferd bis zu einem bestimmten FAN keinen Effekt auf die OMD ausübt (siehe Abschnitt 2.4), was zu beachten ist, wenn Ergebnisse von Verdaulichkeitsuntersuchungen vom Schaf herangezogen werden, die von der empfohlenen Futteraufnahmehöhe nach GfE (1991) abweichen.[28]

Der aus der OMDs abgeleitete OMDh-Wert ist insbesondere dann als sehr zuverlässig anzusehen, wenn damit Unterschiede in der OMDh innerhalb von Futtermittelgruppen festgestellt werden sollen. Dennoch verbleiben für eine Reihe von Konzentrat- und Grobfuttermitteln erhebliche Unsicherheiten hinsichtlich ihres OMDh-Werts. Zukünftige Bemühungen sollten darauf gerichtet sein, allgemeingültige Schätzverfahren für weitere, möglichst große Futtermittelgruppen zu entwickeln, was möglicherweise durch die Berücksichtigung verschiedener Nährstofffraktionen erreicht werden könnte.

3.2.4 Schätzung der OMD aus Faserfraktionen

Die OMD wird maßgeblich vom Gehalt der Futtermittel an Faserfraktionen bestimmt, die Verdaulichkeit der Faserfraktionen insbesondere von deren Lignifizierungsgrad. Andere Nährstoffe oder Nährstoffgruppen weisen in der Regel eine höhere und weniger variable Verdaulichkeit auf. Die Interaktion der „Gerüstsubstanzen“ mit der Verdaulichkeit des Futters wurde schon früh unter Verwendung der CF untersucht (Axelsson 1941, Olsson 1949). Aufgrund ihrer weltweiten Verbreitung in der Futtermittelanalyse kommt der CF eine besondere Bedeutung zu, so dass sie bis heute teilweise noch zur Charakterisierung von Futtermitteln herangezogen wird, obwohl die Grenzen dieser Methode seit langem bekannt sind. So ist der Datenumfang aus Stoffwechselversuchen am Pferd, in denen der Fasergehalt über die CF beschrieben wird, umfangreich. Die Kenntnis des CF-Gehalts ist eine Voraussetzung zur Berechnung sowohl des ME-Gehalts als auch der ME-Verwertung im System des INRA (2015).

Es wurden einige Gleichungen zum Zweck einer möglichen Anwendung in der Futterbewertung mit einer ausreichend großen Datenbasis abgeleitet, die eine große Futtermittelgruppe (Grobfutter, Konzentratfutter) oder Rationen repräsentieren. Sie beschreiben die Abhängigkeit der OMD vom CF-Gehalt (Axelsson 1941; Olsson 1949; Martin-Rosset et al. 1984, zit. bei Martin-Rosset et al. 1994 und INRA 2015; Schulze 1987; Smolders et al. 1990; Zeyner 1995; Fehrle 1999; INRA 2015). Diese Gleichungen können zur orientierenden Einschätzung der OMD herangezogen werden und weisen eine RSD zwischen 2 und 7 %-Einheiten auf.[29] Jedoch unterscheiden sich die verschiedenen Gleichungen teilweise erheblich im Regressionskoeffizienten, der angibt, wie stark die OMD durch die CF-Konzentration beeinflusst wird. Dies ist nicht verwunderlich, da die Faser eine sehr heterogene Zusammensetzung und eine hohe Variation im Lignifizierungsgrad aufweist. Die Lignifizierung wirkt sich bei verschiedenen Futtermitteln unterschiedlich stark auf die Verdaulichkeit der Faser aus, und die CF erfasst nur einen Teil der Zellwandkohlenhydrate und des Lignins im Futtermittel – und darüber hinaus einen variablen. In manchen, besonders aus Getreide und Erzeugnissen aus Getreiden abgeleiteten Gleichungen, deutet der Regressionskoeffizient kleiner −1 an, dass die CF selbst eine geringe Verdaulichkeit aufweist und möglicherweise andere Nährstoffe in ihrer Verdaulichkeit durch die Faser beeinträchtigt werden. Eine Verbesserung der Schätzgenauigkeit der OMD aus der CF konnte erreicht werden, wenn Gleichungen für eng definierte Futtermittelgruppen berechnet oder weitere Inhaltsstoffe berücksichtigt wurden, die dann teilweise allerdings auf einem relativ kleinen Datensatz beruhten.

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[27]Bei Unabhängigkeit der Streuungs- bzw. Fehlerursachen gilt, dass sich der Fehler bei Anwendung beider Schritte aus der Quadratwurzel der Summe der Einzelvarianzen ergibt.
[28]Auf diesen Umstand wird von Smolders et al. (1990) hingewiesen und ein unterschiedliches FAN beim Schaf als mögliche Ursache für nicht mit Chenost et al. (1985) übereinstimmende Beobachtungen angesehen.
[29]Für die Gleichungen von Axelsson und Olsson sind die RSD-Werte nicht bekannt.

Aufgrund der methodenimmanent unzureichenden Differenzierung der Kohlenhydratfraktionen in der CF-Analyse, der relativ großen Streuung in der Beziehung zwischen OMD und CF-Gehalt sowie der teilweise erheblichen Unterschiedlichkeit der diesen Zusammenhang beschreibenden, publizierten Gleichungen ist abzuleiten, dass die CF zur groben Unterscheidung der OMD und des energetischen Futterwerts insbesondere innerhalb einer bestimmten Futtermittelgruppe als Orientierungsgröße herangezogen werden kann. Den Einfluss auf die Verdaulichkeit eines Futtermittels bildet die CF jedoch nicht mit einer für die Futterbewertung erforderlichen Genauigkeit ab. Smolders et al. (1990) konnten zeigen, dass für Grobfutter der CF-Gehalt, für KF der Gehalt an NDF das jeweils bessere Maß zur Schätzung der OMD darstellt. Bei Martin-Rosset et al. (2012) ergab die Schätzung der OMD von Grobfuttern aus dem Gehalt an NDF bzw. Säure-Detergenzien-Faser (ADF, acid detergent fibre) gegenüber dem CF-Gehalt keine höhere Schätzgenauigkeit, während die Schätzung aus dem Gehalt an Säure-Detergenzien-Lignin (ADL, acid detergent lignin) deutlich ungenauer war. Eine Literaturauswertung durch Sales et al. (2013) legt nahe, dass für Grobfutter der NDF-Gehalt hinsichtlich seiner Voraussagekraft dem ADF- bzw. ADL-Gehalt überlegen ist. Es kann aufgrund dieser Arbeiten der Schluss gezogen werden, dass eine Schätzung der OMD aus Faserfraktionen als alleinige Variable, unabhängig von der Bestimmungsmethode, nicht mit ausreichender Genauigkeit möglich ist, wobei noch hinzukommt, dass die verschiedenen Gleichungen nicht zu übereinstimmenden Schätzwerten für die OMD führen. Die Berücksichtigung weiterer Inhaltsstoffe erhöhte die Genauigkeit der für bestimmte Futtermittelgruppen entwickelten Gleichungen jedoch nicht generell. Es ist zwar zu erwarten, dass die Genauigkeit zunimmt, je enger eine Futtermittelgruppe definiert ist. Allerdings leiden solche Gleichungen an der Einschränkung ihres Gültigkeitsbereichs, insbesondere dann, wenn anders zusammengesetzte oder nicht klar zuzuordnende Futtermittel bewertet werden sollen.

Nachfolgend werden in diesem Zusammenhang relevante Ergebnisse aus einer aktuellen Studie dargestellt, der die bisher umfassendste Datenbasis zugrunde liegt und daher eine herausgehobene Bedeutung zukommt (M. Coenen, persönliche Mitteilung). Aus 250 Publikationen des Zeitraums von 1881 bis 2024 konnten 971 OMD-Werte von Rationen und Einzelfuttermitteln entnommen werden, die im Mittel auf 5,9 Einzelbeobachtungen je Ration bzw. Futtermittel beruhen. Allerdings waren die Konzentrationen an NDF, ADF und CF nicht für alle Rationen bekannt, so dass den berechneten Regressionsgleichungen nur zum größten Teil dieselben Rationen zugrunde liegen. Die Mittelwerte und Wertebereiche betragen für OMD 61,3 % (24–99; n = 971[30]), für die Konzentrationen an CP 125 (7–556; n = 967), NDF 532 (30–860; n = 683), ADF 315 (35–596; n = 620) und CF 240 (10–478; n = 538) g/kg DM. Die linearen Regressionsgleichungen zwischen OMD (%) als abhängiger Variablen und NDF, ADF bzw. CF (g/kg DM) als jeweils unabhängigen Variablen lauten:

$$OMD = 90{,}2 - 0{,}057\ NDF \quad (R^2 = 0{,}48,\ RSD = 8{,}0,\ n = 681)$$

$$OMD = 83{,}0 - 0{,}076\ ADF \quad (R^2 = 0{,}37,\ RSD = 8{,}4,\ n = 618)$$

$$OMD = 88{,}4 - 0{,}105\ CF \quad (R^2 = 0{,}62,\ RSD = 8{,}8,\ n = 537)$$

Eine gleichzeitige Berücksichtigung von NDF und CP als unabhängige Variablen spiegelt zwar die negative Wirkung von NDF und die positive von CP wider: $OMD = 85{,}6 - 0{,}053\ NDF + 0{,}022\ CP$ ($R^2 = 0{,}48$, RSD = 8,0; n=581), lässt aber keine genauere Schätzung zu. Die Eingrenzung der Daten auf Grobfutter (Frischgras, Heu, Heulage, Stroh; n = 410) bei gleichzeitiger Berücksichtigung von NDF und ADF führte nicht zu einer deutlichen Reduktion der Streuung (RSD = 7,4, n = 410), wie dies für eine Schätzung erforderlich wäre. Entsprechendes gilt für die Eingrenzung der Daten auf Frischgras und Heu mit den unabhängigen Variablen NDF und CP: $OMD = 75{,}3 - 0{,}042\ NDF + 0{,}036\ CP$ ($R^2 = 0{,}65$, RSD = 6,6, n = 231).

Im Gegensatz dazu stehen die deutlich höheren R^2-Werte aktueller Gleichungen zur Schätzung der OMD von Martin-Rosset et al. (2024), die jeweils gesondert für Heu aus Grünlandwuchs, Grasbeständen und Luzerne berechnet wurden, jedoch auf weniger Datenmaterial beruhen, und bei denen davon auszugehen ist, dass sie einer gewissen Standortspezifität unterliegen. Diese weisen für Grünlandaufwuchs und Grasbestände R^2- und RSD-Werte von 0,68 – 0,87 bzw. 2,4 – 3,5 %-Einheiten auf. Wird die von Martin-Rosset et al. (2024) vorgestellte Schätzgleichung: $OMD = 110{,}8 - 0{,}101\ NDF + 0{,}028\ CP$ ($R^2 = 0{,}86$, RSD = 2,4, n = 32) auf den hier zitierten Datensatz für Gras und Heu angewendet, weist die Beziehung zwischen beobachteten und geschätzten Werten für OMD ein $R^2 = 0{,}42$ und eine RSD = 8,9 (n = 231) auf (M. Coenen, persönliche Mitteilung). Daher kann aufgrund des eingeschränkten Datenmaterials der von

[30]Für einen Teil der Daten wurde die OMD aus der DMD abgeleitet.

Martin-Rosset et al. (2024) angegebenen Gleichungen bei einer allgemeinen Anwendung auf Grobfuttermittel anderer Herkunft nicht mit einer ausreichend genauen Schätzung gerechnet werden. Es ist ergänzend anzumerken, dass angesichts der unterschiedlichen Quellen dieser Daten davon auszugehen ist, dass ein Teil der Streuung in der Beziehung zwischen Faserfraktionen und OMD durch Unterschiede in der Versuchs- und Analysenmethodik bedingt ist.

Der Vollständigkeit halber sei hier das in der Literatur diskutierte Konzept erwähnt, in dem nicht statistische, sondern – wie dies bei Van Soest (1987, Kap. 22.3) ausgeführt und von Zeyner (1995) aufgegriffen wurde – Zusammenhänge zwischen sogenannten uniformen, chemisch bestimmbaren Eigenschaften und der Verdaulichkeit zugrunde gelegt werden. Es wird hierbei im Wesentlichen differenziert zwischen dem Zellinhalt, für den eine hohe und konstante (uniforme) Verdaulichkeit nachgewiesen ist, und der Zellwand, die durch den Grad der Lignifizierung eine variable Verdaulichkeit aufweist. Eine entscheidende Voraussetzung wäre hierfür, dass sich auch die Wirkung des Lignins als eine uniforme, d. h. von der Konzentration und Herkunft unabhängige Wirkung auf den unverdaulichen Anteil der Zellwand mit ausreichender Genauigkeit darstellen lässt. Ob ein solches Konzept ein zukünftiger Weg sein kann, bleibt experimentell weiter zu klären.

Die in dem vorliegenden Abschnitt zusammengestellten Ergebnisse und Befunde bestätigen zum einen die bekannte Tatsache, dass die OMD vor allem durch den Gehalt an pflanzlichen Zellwandbestandteilen bestimmt wird. Andererseits zeigen sie jedoch auch, dass die verbreiteten Verfahren zur analytischen Erfassung derjenigen Bestandteile, die ursächlich für eine verringerte Verdaulichkeit sind, deren Wirkungen auf die Verdaulichkeit nicht ausreichend genau wiedergeben können, so dass eine zuverlässige Schätzung im wissenschaftlichen Sinne als nicht gegeben anzusehen ist und auch in Zukunft nicht erwartet werden kann. Die Vorgänge des Abbaus von organischer Masse im Verdauungstrakt sind so komplex, dass sie sich aus heutiger Sicht quantitativ nur in einem Verdaulichkeitsversuch oder durch eine Simulation von Verdauung und Fermentation erfassen lassen.

3.3 Die Nutzung von Angaben zur Verdaulichkeit der Organischen Masse aus Futterwert-tabellen

In den Futterwerttabellen, die von der Universität Hohenheim – Dokumentationsstelle im Jahr 1995 und von Martin-Rosset im Jahr 2015 (INRA 2015) herausgegeben wurden, finden sich umfangreiche Angaben zur OMD der Futtermittel. Diese stammen bei den DLG-Futterwerttabellen (Universität Hohenheim – Dokumentationsstelle 1995) jedoch nur zu einem geringen Anteil aus Verdaulichkeitsversuchen; stattdessen wurden sie überwiegend von nahezu identischen Futtermitteln übernommen oder mit Hilfe von Regressionsgleichungen geschätzt. Bei INRA (2015) ist die OMD der Grobfutter aus der OMD beim Schaf geschätzt, während die OMD von KF in Verdaulichkeitsversuchen bestimmt wurde. Beide Futterwerttabellen stellen umfangreiche Informationen zur OMD bereit, woraus sich der ME-Gehalt von Futtermitteln mit Hilfe des *dreistufigen Verfahrens* ableiten lässt. Futterwerttabellen stellen damit aktuell eine wichtige Grundlage für die Rationsberechnung dar. Allerdings können nur solche OMD-Werte als gesichert angesehen werden, die durch Verdaulichkeitsversuche bestimmt wurden.

Bei nicht in Tabellen aufgeführten Futtermitteln, deutlich unterschiedlichen Angaben zur OMD, bei Unklarheit, ob das einzuschätzende Futtermittel dem in den Tabellen aufgeführten entspricht, oder bei Fraglichkeit der Richtigkeit des angegebenen OMD-Werts, wenn dieser nicht auf einer Messung, sondern einer Schätzung beruht, wird empfohlen, entsprechende OMD-Werte für Wiederkäuer heranzuziehen (z. B. Universität Hohenheim – Dokumentationsstelle 1997) oder diese mit Hilfe eines *in-vitro*-Verfahrens für Wiederkäuer zu bestimmen (GfE 2023) (siehe Abschnitt 3.2.3). Für Grünlandaufwüchse, Grasbestände und Luzerne ist es hierbei zweckmäßig, die oben angegebene Gleichung [5] zur Schätzung der OMDh aus der OMDs zu verwenden. Erwähnenswert ist, dass die nach Gleichung [5] berechneten OMDs-OMDh-Differenzen für Luzerneheu verschiedener Entwicklungsstadien in guter Übereinstimmung stehen mit denjenigen, die sich aus den jeweiligen OMD-Werten der Futterwerttabelle für Wiederkäuer (Universität Hohenheim – Dokumentationsstelle 1997) und derjenigen für Pferde (Universität Hohenheim – Dokumentationsstelle 1995) ableiten lassen, was die Zuverlässigkeit der Gleichung [5] für diese Futtermittelgruppe unterstreicht. Bereits Fingerling (1940, zit. bei Axelsson 1941) wies darauf hin, dass die Differenz bei Grobfutterleguminosen geringer ist als bei „Wiesenfutter“. Für Grasbestände aus Knaulgras, Wiesenlieschgras, grasreichen Wiesen (wie sie bei Universität Hohenheim – Dokumentationsstelle (1995), S. 64 aufgeführt sind[31]), für Hafer- und Roggenganzpflanzen sowie Getreidestroh (auch aufgeschlossenes) lassen sich aus den Tabellenwerten jeweils mittlere K-Werte im Bereich von 19,6 bis 21,4 (Mittelwert 20,2) ableiten. Generell muss bei solchen Vergleichen (es wurden nur in Verdaulichkeitsversuchen ermittelte Werte herangezogen) beachtet werden, dass es sich hierbei nicht um identisches Material handelt, sondern nur um eine (weitgehend) übereinstimmende Bezeichnung, wodurch nicht unerhebliche Abweichungen

[31]Es wurden die bei Universität Hohenheim – Dokumentationsstelle (1995) für grasreiche Wiesen angegebenen OMDh-Werte mit den OMDs-Werten bei Universität Hohenheim – Dokumentationsstelle (1997, S. 120) für grasreiches Grünland bei gleichen CF-Gehalten verglichen.

bedingt sein können. Für Maissilagen mit einer OMD > 70 % und höherem Kolbenanteil und Stärkegehalten > 25 % kann aufgrund weniger Hinweise ein K-Wert von 12 abgeleitet werden. Bei Futter- und Zuckerrüben, Getreide sowie Konzentratfuttermitteln mit einer OMD > 80 % kann in vielen Fällen von einer ähnlichen OMD bei Pferd und Schaf ausgegangen werden, was durch die Untersuchung von Martin-Rosset und Dulphy (1987) mit einem hochverdaulichen KF-Gemisch gestützt wird. Eine Ausnahme bildet Hafer, der bei Pferden eine geringere OMD aufweist. Für Erzeugnisse und Nebenerzeugnisse der Getreide- und Ölsaatenverarbeitung mit geringerer OMD sowie für Körnerleguminosen zeigen sich jedoch variable Werte für die OMDs-OMDh-Differenzen, so dass sich für diese Futtermittel aus ihrem OMDs-Wert offensichtlich nicht direkt auf die OMDh schließen lässt.

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