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- *Abstracts (Kurzfassungen der Originalmitteilungen)*
- *Workshop-Beiträge*

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

J. Aschenbach
Chairman

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Review lecture

The manuscript of the review lecture represents the translation of the original published book chapter "Tierbasierte Bioökonomie" of the book "Das System Bioökonomie". The references in the original text to other chapters of the book as well as the book chapter numbering were retained to assure comprehensibility (Thrän, D., Moesenfechtel, U. (Hrsg.) (2020): Das System Bioökonomie, Springer-Verlag, https://doi.org/10.1007/978-3-662-60730-5).

Livestock-based circular economy: Perspectives and conflicting goals in the role of livestock in the agricultural production of food of animal origin

Nutztierbasierte Bioökonomie: Perspektiven und Zielkonflikte in der Rolle der Nutztiere bei der agrarischen Erzeugung von Lebensmitteln tierischer Herkunft

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5.1 Introduction

As the world population continues to grow unabated, the demand for food will continue to rise (FAO 2018; Searchinger et al. 2018; Smith 2018). This also involves a massive increase in global demand for food of animal origin, especially meat, which is considered a "traditional symbol of prosperity". On the other hand, there are already more than 800 million people (about 11 % of the world's population) suffering from chronic hunger, about 2 billion people have to live with food insecurity (FAO 2019). According to FAO recommendations (FAO 2013), adults should consume about 20 g of protein of animal origin per day to have a balanced diet. This amount is reached as a global average (23.9 g/day), but it varies between 1.7 (Burundi) and 69 g (USA; Germany: 52.8 g/day). This comparison reveals the enormous inequality in the supply of the world's population with quality food, which is likely to worsen in the future.

Moreover, livestock feeding takes up a significant proportion of agricultural land. While in 1970, about 0.38 ha were available per inhabitant at a global scale, the value had decreased to 0.24 ha in 2000, and in 2050, it will probably have dropped to only about 0.15 ha. In Germany, currently about 0.22 ha are available per inhabitant. Smith (2018) considers this to be one of the greatest challenges facing humanity. Accordingly, the keeping of livestock for the purpose of producing high-quality food, especially food containing protein, is increasingly being discussed with view to the associated consumption of resources (land, water, energy) and the accompanying emissions, particularly in the current "agri-food system" (Diaz 2019; Flachowsky et al. 2019, for example). On the other hand, livestock and the food of animal origin produced thereof are also an important economic factor, especially in industrialised countries. Germany, for example, has more livestock than people (12 million cattle, 25 million pigs and almost 180 million poultry alone). They generate about twice as much agricultural production value in the form of raw milk, eggs and live animals for slaughter than crop production (in Germany, 53 vs. 23 billion euros in 2018; Kohlmüller and Koch 2019).

The present article examines the agricultural background of current livestock farming. Special attention is paid to the intensive interconnectedness of the mass flows between livestock farming and the agricultural production of plant biomass, including the return of large quantities of by-products from the industrial processing of primary plant products to the agricultural cycle of materials via livestock feeding. This leads to limitations, but also creates new opportunities regarding the ways agricultural animal husbandry could respond to the current and future challenges in Germany in the context of livestock-based bioeconomy.

5.2 System description

5.2.1 The role of livestock in the agricultural food production system

In the system of agricultural food production, livestock play a central role as biomass transformers. While fully maintaining the mass balances, the ingested biomass is transformed, via digestion and metabolism, into excreta (faeces, urine), gases (CO_2, with ruminants also CH_4) as well as into an increase in body substance, milk or eggs – i.e. mainly into the build-up of proteins, fats and carbohydrates.

As is shown in Fig. 5.1, only a small part (approx. 10 to 20 %) of the agrarian plant biomass reaches human consumption at all. The main reason for this is the fact that most of the agricultural biomass is, in principle, unfit for human consumption, such as biomass from grassland or from intermediate crops. This type of biomass is an inevi-

table part of the overall agricultural production of biomass, as the sustainable cultivation of food-supplying crops requires a crop rotation that also includes non-edible intermediate crops. In addition, a significant share of agricultural land can only be used as grassland, for geographical or environmental reasons (topography, remoteness, rainfall, temperature, groundwater, proximity to water sources, etc.). But even with food-supplying plants (e.g. cereals), not even half of the biomass harvested is suitable for further use as food (e.g. grains versus straw). This biomass from grassland, intermediate crops and crop residues accounts for more than half of the total agricultural biomass and serves as the primary feed basis for ruminants in particular. However, plant breeders have made little effort to develop the feed value of this type of biomass. Progress in this area will be of great importance for the livestock-based bioeconomy in the future. Great hopes are placed in modern breeding methods such as genome editing of feed value-determining traits of crops in particular (National Academy of Sciences Leopoldina et al. 2019). The industrial processing of plant products into foodstuffs such as flour, sugar or cooking oil, or energy sources such as biodiesel and bioethanol, or into other valuable industrial materials, produces even more by-products in considerable quantities. Often, the by-products significantly exceed the quantity of the actual target product: for soy, for example, the ratio is 2:1, for rapeseed 1.5:1. These residues from the industrial processing of plant products usually serve as high-quality feedstuffs that are not, or to a limited extent only, fit for human consumption. They are mainly used for feeding poultry, pigs and high-yielding ruminants and account for almost half of the compound feed traded globally. Thus, the "modern" production of food of animal origin is fundamentally based on the intensive interconnectedness of the biomass processing industry with agriculture.

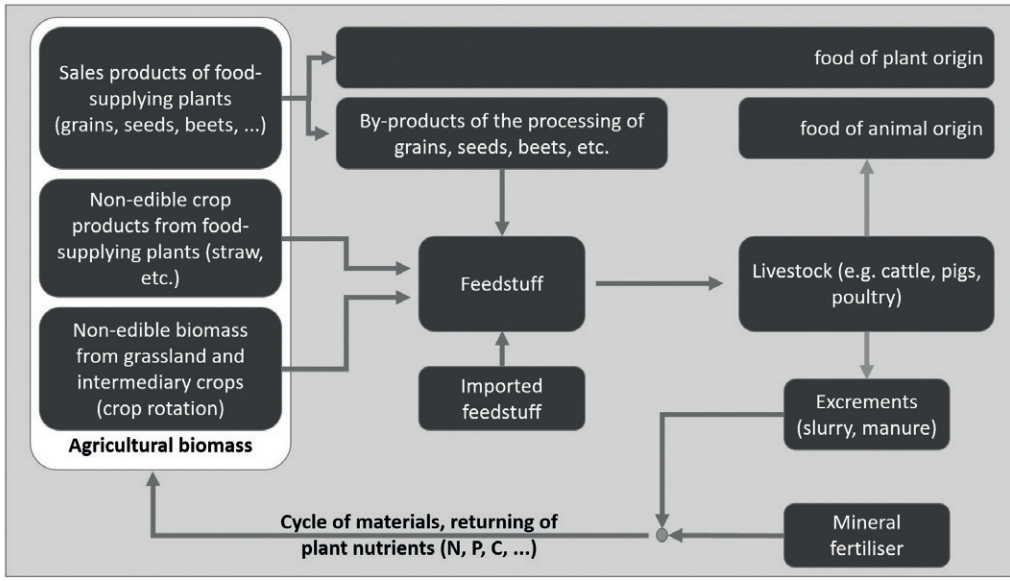

Fig. 5.1 Schematic flow of biomass and the contained plant nutrients between agricultural crop and livestock production. (Source: authors' own illustration)

The feeding of livestock generates excrements which, in the form of manure, return a large proportion of the plant nutrients fixed in the biomass (nitrogen, phosphorus, etc.) to the agricultural land in a highly available form. Through this, livestock are fundamentally involved in maintaining the agricultural nutrient cycle and can replace mineral fertilizers to a considerable extent. Therefore, they need to be included in the bio-economic assessment of the agricultural production of biomass as a general rule. Conversely, a livestock-based bioeconomy would be incomplete without considering its fundamental effects on the agricultural production of plant biomass.

A prerequisite for the efficient transformation of biomass through livestock is that the feed is of high quality and fully balanced with regard to all essential nutrients. For this purpose, livestock farmers often purchase additional feedstuff, for example protein feeds such as soya and rapeseed meal or mineral feeds containing phosphorus, for example. These feedstuffs, too, are subsequently transformed into manure by the livestock. This indirect import of plant nutrients through purchased feed need not be viewed negatively per se, as it can compensate for the export of

plant nutrients through the sale of agricultural products.
Only when import rates are high will the nutrient cycle between animal husbandry and primary plant production become unbalanced. Where this threshold lies depends on the efficiency with which the plant nutrients bound in the feed biomass are transformed into sales products of animal origin. Apart from the quality of the biomass available as feed, this is also a question of the livestock species, the kind of output (meat, milk, eggs), the performance level and, in particular, the conceptual design of the livestock feeding regime which implements the current state of knowledge of animal nutrition.

Protein source	Level of performance (per animal per day)	Dry matter intake (kg per animal per day)	Ratio of roughage[1] to concentrates[1] (in % of DM)	Edible protein (per g/animal per day)	Land footprint (m^2/kg edible protein)[4,5,6]	Water footprint (m^3/ kg edible protein)[6]	Carbon footprint (kg CO^2equ/ kg edible protein)[6]
Milk	5 kg	10	95/5	163	33-135	16,0	50
	10 kg	12	90/10	323	22-88	10,9	30
	20 kg	16	75/25	646	15-68	10,5	16
	40 kg	25	50/50	1292	15-70	12,3	12
Beef	500 g LWG	6,5	95/5	48	72-295	34,0	110
	1000 g LWG	7,0	85/15	95	41-180	24,7	55
	1500 g LWG	7,5	70/30	143	35-155	24,5	35
Pork	500 g LWG	1,8	20/80	45	36-176	35,8	16
	7	2,0	10/90	63	30-148	31,3	12
	10	2,2	0/100	90	24-120	26,1	10
Poulty	40 g LWG	0,07	10/90	4,8	14-68	14,4	4
	60 g LWG	0,08	0/100	7,2	12-60	11,8	3
Eggs	50 % LP	0,10	20/80	3,4	28-122	26,5	7
	7	0,11	10/90	4,8	26-105	22,5	5
	9	0,12	0/100	6,2	20-95	20,8	3

[1)] Roughage: fresh or preserved biomass from grassland and intermediary crops, rich in fibre, non-edible Concentrates: high-quality mixtures of grains and by-products, low in fibre, partially edible

[2)] Live weight gain per day

[3)] Laying performance

[4)] Some authors calculated LFP without permanent grassland in non-ruminant feeding

[5)] High fluctuations due to different yield levels (Flachowsky et al. 2017) and different shares of by-products in the rations

[6)] Values can be massively influenced by reproductive performance, diseases, animal losses and other factors (Özkan et al. 2016)

Fig. 5.2 Feed intake, edible protein yield and footprints (FP) per kilogram of edible protein of animal origin for different animal species/categories and different performance levels (feed data expressed as dry matter (DM)). (Source: authors' own illustration based on Flachowsky et al. 2017)

5.2.2 Assessment of the transformation of biomass into edible protein

The assessment of the consumption of resources by livestock and their environmental impact is usually made in a generalised manner in relation to a sales product (for example, one kilogram of meat). However, this perspective severely limits the differentiated consideration of the diverse livestock groups (e.g. poultry, fish, pigs, ruminants) and output categories (meat, milk, eggs) as well as the high variability of output levels. For this reason, various authors (for example Flachowsky and Kamphues 2012; Nijdam et al. 2012) have attempted to compare the wide range of production methods of food of animal origin by means of objective parameters. The amount of edible protein of animal origin that ultimately reaches the consumer is particularly suitable as a common basis. Species, production

specialisation, performance level and other factors have a considerable influence on the formulation of the ration and the feed intake of livestock as well as the amount of edible protein produced daily. Among other things, ruminants (e.g. cattle, sheep, goats) are able to use cell wall-rich feedstuffs such as grass or straw for energy production with the aid of the microorganisms living in their forestomach system (Sect. 5.2.1). Therefore, their rations contain far more non-edible biomass than those of non-ruminants such as pigs or poultry (Fig. 5.2). Besides, the microorganisms of the forestomach system use non-protein nitrogen compounds (e.g. urea) to produce large quantities of high-quality protein. This means that ruminants are largely independent of the supply of protein via the feed ration, or even completely independent at low performance levels, and are therefore on principle no food competitors of humans. Increased methane formation with a relatively high greenhouse gas factor (GHG; about 23 × CO_2; IPCC 2006) is one of the negative effects of this microbial colonisation of the forestomachs.

High-yielding dairy cows produce the largest quantities of edible protein (approx. 1 kg/day). However, in terms of live weight (LW), laying and growing poultry are clearly superior to dairy cows. The lowest protein yield per kg LW is produced by growing ruminants, followed by fattening pigs (Fig 5.2). For each kilogram of edible protein, the land, water and carbon footprints (FP) become smaller at higher outputs (see also Niemann et al. 2011, Windisch et al. 2013), while the amount of concentrates required increases (Fig. 5.2). The high ranges of variation in the land FP result from different influencing factors in the corresponding calculations (see footnotes).

With regard to water FP (WFP), there is currently a controversial debate. For the calculations in Fig 5.2 only "blue" water was considered, i.e. water from reservoirs, lakes or rivers. It is the only water supplied to plants by human activity, and the consumption is measurable (Tom et al. 2016). According to the data shown in Fig 5.2, beef production is particularly costly in terms of land and water FP. It also has by far the highest carbon FP per amount of protein produced. On the other hand, this is also the branch of production that can use the largest quantities of high-fibre, non-edible biomass.

Another topic to be critically discussed in connection with the production of food of animal origin is the competition for food between humans and animals. According to FAO statistics, about 85 % of the world's soya harvest and about one third of the world's cereal harvest is used for animal feed. However, a high proportion of this biomass could also be consumed directly by humans. In view of the increasing limitation of arable land on which this biomass is produced and the rising world population, the competition for use between feed and food will increase sharply in the future. Particularly affected are those livestock that consume a lot of "concentrated feed" – for example, non-ruminants such as pigs, fattening poultry and laying hens (Fig. 5.2), i.e. precisely those production sectors that are characterised by a high transformation efficiency in the production of edible protein and comparatively low footprints. This reveals a fundamental dilemma of livestock feeding: high efficiencies and low environmental impacts require predominantly high-quality feed, which in turn increases the food competition with humans. While non-edible biomass generates more emissions, limits the performance level of the animals due to the lower feed quality and is therefore less efficient overall, it can be transformed into edible protein without any food competition. Against this background, the often-criticised production of beef and milk is certainly sustainable, especially since the footprints of milk production hardly differ from those of monogastric livestock.

In the future, increasing food competition will promote livestock systems with a high potential for utilising non-edible biomass. This includes ruminants, which can digest non-edible biomass by means of their forestomachs, but also monogastric livestock (pigs, poultry; see also Hendriks et al. 2019), provided they are fed, for example, lower-quality by-products of the industrial processing of primary plant products (e.g. from the processing of cereals or rapeseed).

Fig. 5.3 shows the proportion in various feedstuffs that is edible for humans (human edible fraction, hef). The respective hef data are to be interpreted as ranges of values, since no clear boundary can be drawn between edible and non-edible biomass. Many primary plant products such as cereals, maize, soya etc., which are often generally referred to as "foodstuffs", contain considerable amounts of components worth feeding.

Feedstuff	hef (% DM)		
	Low	medium	high
Barley	40	65	80
Maize	70	80	90
Wheat	60	80	100
Soya beans	50	92	93
Rapeseed	30	59	87
Wheat bran	0	10	20
Maize silage	19	29	45
Others[1]	0	0	0

[1)] Other by-products (e.g. dried pulp, brewer's grains, distiller's wash) and roughage (e.g. grass, silage from grass and legumes, hay, straw)

Fig. 5.3 Human edible fraction in different feeds (hef, in % of DM). (Source: authors' own illustration based on Ertl et al. 2015)

Such data help to make the discussion about food competition between humans and animals more objective. Van Zantem et al. (2016) propose to specify the production of edible animal protein per hectare of agricultural land, taking into account the use of by-products. Along similar lines, Nie et al. (2018) have developed a so-called food energy water-nexus for various feed or animal husbandry systems. Overall, the data show the great potential of by-products from the industrial processing of plant raw materials and their growing importance in the production of food of animal origin.

5.3 Prospects for a more efficient and sustainable production of food of animal origin

Improving the transformation efficiency of biomass in the system of modern agricultural livestock production has two main objectives:

a) to minimise the consumption of biomass for non-productive life processes in relation to total consumption (see also Niemann et al. 2011) and b) to optimise the efficiency of the transformation within the productive processes. Even non-productive life processes, such as maintenance metabolism, consume energy and nutrients and cause emissions. Breeding animals for higher performance has consistently reduced the share of maintenance requirements in total nutrient requirements. However, the gain in efficiency follows a dilution function and is therefore degressive. In modern high-performance breeds, the increases in transformation efficiency to be expected from simply breeding for even higher performance are relatively small. The situation is different when it comes to feed costs and the environmental impact of maintaining the livestock systems. For example, a female bovine must be raised for 24 to 30 months before it can begin to produce milk. Given that the average number of productive years of dairy cows in Germany is less than four, optimising the rearing period and increasing the lifetime yield, for example by increasing longevity, has a significant impact on the transformation efficiency and environmental impact of the entire production system. This principle applies to all types of livestock husbandry. Approaches to improve the utilisation of biomass for productive life processes (growth, production of eggs and milk) basically cover two areas, a) the digestive tract and b) the metabolism beyond the intestinal barrier.

As is shown in Fig. 5.4, the ingested biomass is broken down by the body's own digestive enzymes and microorganisms into low-molecular nutrients, which are supplied to the metabolism beyond the intestinal barrier through absorption. The extent to which this is achieved depends on how well the composition of the feed biomass matches the digestive capacity of the livestock species concerned. For example, ruminants can digest high-fibre, non-edible biomass well, whereas monogastrics can only do so to a limited extent (Sect. 5.2).

Beyond the intestinal barrier, the composition of the absorbed nutrients differs only marginally between the various livestock species and also humans. The subsequent metabolic processes are also very much evolutionary conserved in their biochemical nature. It is certainly possible to shift the regulation of metabolism through breeding or pharmacological interventions – such as the application of growth hormone – and to steer the flow of absorbed nutrients

in a certain performance direction. However, the efficiency of the processes involved is largely determined and will be achieved when all essential nutrients (limiting amino acids, for example) are available in the metabolism in an optimal ratio.
The actual efficiency of nutrient transformation in the metabolism therefore depends primarily on the extent to which the optimal supply of the metabolism with the various nutrients is achieved by appropriate feeding. These interactions between the digestive tract and metabolism provide several starting points for increasing the efficiency and sustainability of the production of food of animal origin.

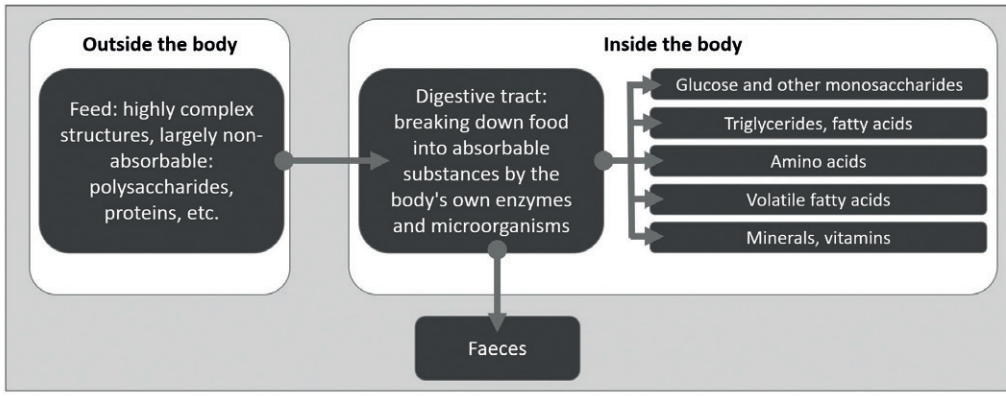

Fig. 5.4 Schematic of the flow of biomass from feed via the digestive tract into the metabolism. (Source: authors' own illustration)

5.3.1 Increasing the quantity and quality of forageable biomass

A conservative approach to increasing the quantity of biomass aims at minimising losses along the route from the field through harvesting and conservation (drying, ensiling) to feeding. With regard to the quality of biomass in terms of its suitability as animal feed, there are three main limiting aspects:
(a) the presence of antinutritive or toxic ingredients,
(b) high levels of components with a low digestibility; and
(c) the coupling of high and low feed value sub-fractions in the same feed material.
Antinutritive or toxic ingredients in otherwise high-quality biomass are very significant bottlenecks regarding its use as potential feed. For example, former rapeseed varieties naturally contained such high levels of toxic or highly antinutritive substances (erucic acid in the oil, glucosinolates in the water-soluble components of the dry matter) that it could not be used at all, or to a very limited extent only, both as food (edible oil) and as animal feed (e.g. by-products of the extraction of rapeseed oil). However, the massive reduction of these critical ingredients through plant breeding about two decades ago led to a triumphant success of the protein-rich by-products of oil extraction from rapeseed in livestock feeding and fundamentally changed the profile of circular economy of rapeseed. Cotton seed is another very interesting candidate in terms of quantity as regards the diminution of strongly antinutritive ingredients, especially the so-called gossypol. Significant breeding and genetic engineering successes have already been achieved in reducing the gossypol content, which have considerably expanded the possible uses of cotton seed as a high-protein feedstuff in livestock feeding (for example Sunikumar et al. 2006). These examples show the great potential of plant breeding and genetic engineering to improve feed quality and thus to specifically influence the bioeconomy of the connection between agricultural crop and livestock production, including the by-products of the industrial processing of agricultural biomass (see also Flachowsky 2013; Flachowsky and Meyer 2015; NASEM 2016; Chap. 3).

Biomasses with very high contents of lignocellulose such as straw are only suitable as animal feed, if at all, for ruminants, because lignin can hardly be degraded in the forestomachs and also strongly impedes the microbial digestion of the associated cellulose structures. This caging effect of lignin is the reason why wood itself is not suitable as feed for ruminants, even though the cellulose per se would have a considerable nutritional value. The degradation of lignin structures in biomass would thus massively expand the portfolio of potential feeds, both quantitatively

and qualitatively (Blümmel et al. 2018). The feasibility of such approaches was demonstrated decades ago (for example, Kerley et al. 1985) and also temporarily implemented in practice with cereal straw, for example (Sundstol and Owen 1984; Flachowsky 1987), although technical and economic difficulties at the time prevented widespread use. However, with new developments in the circular economy of lignocellulosic biomass such as wood, these largely forgotten approaches could become interesting again. Another approach would be to separate the valuable from the value-reducing sub-fractions of the biomass. Options range from the mechanical coarse separation of plant material (for example leaves versus stems) to the extraction of high-quality protein from press juices of green biomass or from residues of (bio-)technological processes (for example from distillers grain solubles of bioethanol production). In principle, this is nothing other than the consistent extension of food and feed technology processes to non-edible biomass or by-products.

5.3.2 Expanding the digestive capacity of livestock

Apart from the volume of the digestive tract, the limitation of the digestive capacity of humans and animals is based mainly on a limited endowment with endogenous digestive enzymes. The supply of exogenous enzymes is an established method to widen this bottleneck. It can be used to strengthen existing capacities, with proteases supporting protein digestion, for example, or to introduce fundamentally new biological degradation capacities into the digestive tract (Chap. 6). A prominent example are phytases from biotechnological production (usually based on genetically modified microorganisms). They compensate the inability of animals and humans to endow their body with digestive enzymes to release phosphorus from the phytic acid found in grains and seeds. However, the list of enzymes available so far is still very limited. This area holds an enormous potential for innovation, as it would be possible to adapt the digestive capacity of livestock to a variable composition of biomass through the targeted supplementation of enzymes. Of particular interest would be additives that enable monogastric livestock to digest non-starch polysaccharides enzymatically. Overall, the development and large-scale production of enzymes as feed additives for livestock nutrition holds an enormous potential of expansion for the bioeconomy of microorganisms (Chap. 6).

An indirect improvement of the digestive capacity is achieved by all measures that improve gut health (application of organic acids, probiotics, phytogenic additives, exogenous enzymes to break down non-starch polysaccharides) (Gonzalez-Ortis et al. 2019).

One of the visions for future ruminant husbandry is shifting the microbial degradation of cellulosic dietary fibre from acetic acid fermentation towards propionic acid. In addition to reducing the emission of climate-damaging methane, this would result in a massive increase in the yield of nutritional energy from a non-edible feed substrate, as the calorific value of the methane remains chemically bound in the propionic acid and is available to the animal's metabolism as absorbable nutritional energy. However, microbial digestion of dietary fibre is closely linked to the formation of methane. Measures to reduce the formation of methane – for example, by means of broadly acting feed additives (herbal extracts, antimicrobial substances, etc.) – therefore often also inhibit fibre digestion and thus reduce the feed intake of the animals. Recently, however, additives have been developed that highly specifically block only the last enzymatic step of methane formation and therefore cause less collateral damage to the fermentation ability of the forestomachs (Duin et al. 2016).

5.3.3 Optimising the metabolism

As has been described earlier, this aspect is not about increasing the efficiency of individual metabolic processes, but about avoiding an inadequate supply of nutrients, including both deficiencies and surpluses. This requires very precise concepts for determining the metabolic demand for nutritional energy and nutrients as well as the delivery capacity of biomass for the respective livestock species and categories. With regard to meeting the demand for essential ingredients, plant breeding can certainly make a contribution, for example by increasing the shares of limiting essential amino acids in plant protein (see also Flachowsky and Meyer 2015; NASEM 2016). The targets for plant breeding can only be formulated relatively roughly, though, as the specific demand patterns vary according to animal species, kind of output and performance level. Fine-tuning of the supply of limiting nutrients, on the other hand, is achieved by supplementing the feed with pure, synthetically or biotechnologically produced substances (e.g. crystalline amino acids, vitamins). They play a fundamental role in modern diet formulation and are indispensable instruments for minimising environmentally relevant emissions from livestock farming. The consistent supplementation of the feed of pigs and poultry with limiting amino acids, for example, allows for a reduction of

the crude protein content of the feed by several percentage points and, as a consequence, lowers nitrogen emissions by approximately one third compared to rations without amino acid supplementation (Flessa et al. 2012; Sajeev et al. 2018).

5.3.4 Novel types of biomass and livestock

New (bio-)technological processes will generate new types of residues, which may well be suitable as feed for livestock. With view to a cascading use, the nutritional potential of these by-products must always be taken into account and, if necessary, already considered in the primary production process. In the future, more and more plants will be cultivated which have so far been hardly or not at all considered for livestock nutrition. Many plants from tropical and subtropical regions, for example opuntia, have high potentials for animal nutrition. In this context, plant biomass of aquatic origin appears to be particularly interesting, as it does not compete with other terrestrial biomasses for limited agricultural land. Examples of such aquatic biomasses are macroalgae and microalgae. The latter are mainly discussed for the extraction of protein (partly also fat) and will be examined in detail elsewhere (Chap. 7). Macroalgae, on the other hand, provide carbohydrates, above all, and in a form that is largely enzymatically indigestible for terrestrial livestock (Brugger et al. 2019). Not even fish (for example in aquaculture) possess suitable endogenous digestive enzymes. In contrast, aquatic molluscs do possess digestive enzymes adapted to the specific carbohydrates from macroalgae (Michl et al. 2014). This example shows that the use of novel types of biomass is often accompanied by the search for novel animals capable of digesting this biomass – such as molluscs as potential transformers of macroalgae.

This basic principle of matching as closely as possible the characteristics of the biomass and the digestive capacity of the transformers in question also applies to the discussion of insects as a potential novel type of livestock. Many of the insects currently being considered for use are food competitors of both humans and conventional livestock (EFSA 2015). They generally require highly digestible biomass for efficient transformation, indicating a limited digestive capacity analogous to monogastric livestock. In fact, the digestive capacity of individual insect species remains largely unexplored, although this knowledge is essential for the sustainable use of insects as novel transformers of biomass.

In principle, even excrements can be considered potentially usable biomasses. The feeding of nitrogen-rich excrements, for example sterilised poultry manure, has a certain tradition as a source of crude protein for the microorganisms in the forestomachs of ruminants. Especially in regions with roughage low in crude protein (e.g. grassland in the tropics), these microorganisms benefit from the additional supply of nitrogen, which they can use to build up microbial protein, which in turn serves the ruminant as an important source of protein. Another example is the use of manure to breed black soldier flies, which can then be used as protein feed for livestock. However, the use of excrements in animal feed is prohibited in many regions for reasons of hygiene and food safety. In the European Union, for example, excrements in livestock feed are among the list of prohibited substances.

5.4 Conflict of objectives

As outlined above, the global demand for food of animal origin is expected to increase massively. This will create an enormous future market for animal production – especially for production methods with a high transformation efficiency (for example poultry meat). Associated relative advantages regarding the environmental impact (Fig. 5.2) will accelerate this trend. Thus, it is not surprising that the consumption of poultry meat has increased massively in recent decades and has almost overtaken pork as the global leader in meat consumption (OECD/FAO 2018). However, these highly efficient production methods are the ones causing the highest food competition. The core of the conflict of objectives between production efficiency, environmental impact and food competition lies primarily in the shrinking availability of agricultural land (Sect. 5.1), which is used for the production of food, feed, energy sources and other industrial valuable substances of plant origin. The circular economy of agricultural plant and animal production and the downstream industrial processing of the respective products are inextricably linked via the factor of agricultural land and thus enter into direct competition with each other. The production of animal feed is not fundamentally at stake, though. In the future, considerable amounts of non-edible biomass will continue to accrue from grassland and from co-products from cultivated plants, intermediary crops and as by-products of the industrial processing of plant products, which can be transformed into high-quality foodstuffs by means of appropriate production systems (above all ruminants). The resulting manure supports the agricultural cycle of plant nutrients and thus indirectly promotes the production of food of plant origin. Alternatively, the non-edible biomass

could also be utilised energetically in biogas plants and the residues returned to the cycle of plant nutrients in the same way as the excrements of livestock. But apart from losing high-quality food, biogas plants work much more slowly than the forestomachs of ruminants. The half-life of microbial degradation of organic matter in the forestomachs of ruminants is less than one day, but in biogas plants it takes about 5 days (ranging between 3 days and 2 weeks, depending on the quality of the fermentation substrates (Dandikas et al. 2018)). The disadvantages of the targeted use of non-edible biomass lie in the lower transformation efficiency and the associated higher emissions (e.g. methane). Other negative effects that are often mentioned, such as the consumption of land and water, do not have an impact here, as long as only the use of non-edible biomass accruing anyway is concerned and no additional agricultural land (especially arable land) is used for the cultivation of animal feed. However, the quantities of food of animal origin that can be produced in this way lie significantly below the current and particularly the future demand and would require a massive change in dietary habits (for example, Schader et al. 2015).

Apart from the limited availability of non-edible biomass, another factor limiting its transformation into food of animal origin are food safety aspects. In principle, this applies to waste of any kind. In contrast to by-products from the industrial processing of agricultural raw materials, waste materials are indeterminate and uncontrolled in their origin. Accordingly, they hold the risk of entering undesirable substances into and threaten the hygienic standard of the food chain. A similar assessment can be made for excrements such as those considered for producing insects (for example, black soldier fly on manure) (see also EFSA 2015). As a matter of principle, non-edible biomass, too, should come exclusively from regular agricultural land or the controlled processing of its products. This is the only way to guarantee a high level of food safety.

Another conflict of objectives touches on the ethical aspects of livestock production. Livestock are increasingly perceived as fellow creatures, with people questioning the animals' "exploitation" for their own nutrition purposes. Accordingly, the discussion about artificial meat, among other things, has gained considerable momentum. No livestock need to be killed for it, the cell cultures grow very efficiently, there is no slaughter waste, and a very high level of hygiene can be obtained. However, the latter requires antibiotics, which again pose a risk to food safety. The real bottleneck in this production of valuable food lies in the need for the highest quality nutrients to "feed" the cell cultures, though. These nutrients have to be generated through primary agricultural production and/or costly industrial processing. This means that such production methods inevitably come into conflict with the objectives of environmentally relevant emissions, consumption of agricultural land and competition for food. The extent to which they actually promote circular economy in comparison to conventional livestock farming can only be determined by means of comprehensive live cycle assessments.

5.5 Prospects

Apart from a growing population and rising temperatures (IPCC 2019), increasing urbanisation and growing global affluence are considered to be major changes that will strongly influence and, in part, challenge the current role of livestock in providing food to humans in the future (Mottet et al. 2017). The result is a call for more livestock products and an expansion of agricultural land, partly through irrigation using non-renewable water supplies and through deforestation. Further, the food competition between humans and livestock – especially non-ruminants – will continue to intensify (Bryan et al. 2015) and climate-relevant greenhouse gases caused by livestock production will continue to increase (Lesschen et al. 2011). All these developments are heading in an unsustainable direction and require countermeasures. In essence, the aim is to produce the unavoidable additional demand for food associated with the growing number of people with fewer resources and emissions overall ("sustainable intensification") and, in addition, to search for alternatives to conventional food production. Plant breeding – in particular on the basis of genome editing – is of primary importance here (e.g. adaptation to higher temperatures, drought, higher atmospheric CO_2-concentrations, salt water, etc.) (Weigel and Manderscheid, 2012; NASEM 2016; Bailey-Serres et al. 2019; National Academy of Sciences Leopoldina et al. 2019), followed by innovative cultivation techniques, plant protection measures, as well as harvesting and preservation methods (HLPE 2019).

But the need for sustainable intensification will also require considerable changes in the way livestock are kept. First and foremost is the avoidance of food competition between humans and livestock, and in two directions: it is necessary to map out the perspectives and potentials of ruminants using grassland, co-products from cultivated plants and intermediary crops in the course of crop rotation as well as by-products. But monogastric livestock have considerable potential in the utilisation of non-edible biomass, too. Pigs, in particular, have been fed almost exclusively on non-edible biomass since their domestication and have only recently become a food competitor to

humans as a result of breeding for high performance. These “archaic” abilities of monogastric livestock need to be reactivated.

Non-edible biomass from crop production must be regarded as a valuable raw material to an even greater extent than in the past. As it will be the basis for the production of food of animal origin in the future, it must also be intensified in a sustainable manner as regards quantity and quality. Here, too, plant breeding and genetic engineering (e.g. with regard to antinutritive or toxic ingredients), cultivation techniques and, in particular, innovative harvesting and preservation methods are required to maintain the feed value of the usually perishable biomass. In addition, the by-products of the processing of plant products must be consistently returned to the feeding cycle.

However, the demand for a strict avoidance of food competition between humans and livestock will only be implemented to a limited extent in the foreseeable future. This is due to the fact that the expected demand for food of animal origin can hardly be met on the basis of non-edible biomass alone. The price for this food would be very high and the supply of food to people would follow the global wealth gap even more than before. As is shown in Fig. 5.5, these socio-economic conditions are just as important for the kind and scope of future animal production as are resource consumption, environmentally relevant emissions and, increasingly, ethical aspects. It is therefore to be hoped that people's nutritional habits will change in the future and that a limited intake of food of animal origin will become generally accepted. In order to support this development, a consistent further development of life cycle assessments, which allow for objective comparisons of the overall effects of measures and alternative proposals for the production of food, is required.

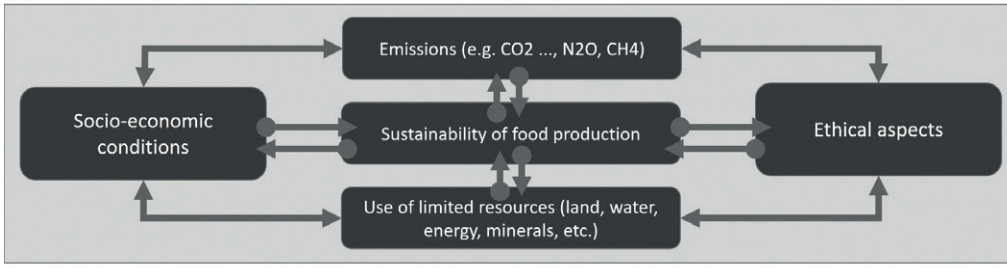

Fig. 5.5 Sustainability in the production of edible protein of animal origin as a balance between limited available natural resources, emissions, socio-economic conditions and ethical aspects. (Source: authors’ own illustration)

Finally, the question arises whether the future will bring a form of agriculture without livestock. After all, farm animals are no longer needed in large numbers as working animals. Moreover, the complete renunciation of food of animal origin would eliminate all ethically motivated reservations concerning livestock. For humans, this would not be a fundamental nutritional problem either, as long as other high-quality foods and, if necessary, supplements such as amino acids or vitamins are always sufficiently available. With view to the enjoyment value, plant-based imitations already exist or have long had a firmly anchored cultural identity (for example, tofu versus cheese). They are complemented by products from new technological approaches, which are referred to collectively as cellular agriculture or meat alternatives and are already being discussed at scientific level (for example, Grieve et al. 2019). However, such products tend to be relevant for industrialised countries only, while the nutrition of underdeveloped regions will continue to depend largely on livestock. Herds of cattle and flocks of sheep, for example, basically do not require any technical infrastructure such as roads, electricity, and so on. The question of the dispensability of livestock rather touches on the basic principle of primary agricultural production based on crops. In addition to the actual “food” components, these always contain considerable amounts of non-edible biomass as well, which must be degraded to plant nutrients and returned to the agricultural land (Fig. 5.1). Livestock perform this function in a way that has been established for thousands of years, generating highest-quality food for humans (and they also used to serve as working animals). The abandonment of livestock without alternative would therefore not only result in an absolute loss of food but would also reduce the productivity of crop cultivation or require an increased use of mineral fertilisers.

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Abstracts

1.

Influence of different amounts of black soldier fly larvae (BSFL) in the ration on nutrient and energy utilization and growth of broilers

Einfluss unterschiedlicher Mengen an ganzen Schwarzen Soldatenfliegenlarven (BSFL) in der Ration auf die Nährstoff- und Energieverwertung und das Wachstum von Broilern

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Insect larvae are part of the natural diet of poultry. In recent years, the meal of the Black Soldier Fly Larvae (BSFL; *Hermetia illucens* L.) has been suggested as an alternative source of protein to soybean and hence, as a potential ingredient for chicken feed (1). The inclusion of BSFL-meal in poultry diets implies the extraction of protein and fat from the larvae. Instead of the meal form, whole BSFL could be fed to chickens directly without the necessity of expensive processing technology. Therefore, the objective of this study was to investigate whether and to what extent the inclusion of unprocessed BSFL in broiler diets influences nutrient and energy intakes as well as growth performance of broilers.
Methods: A total of 252 newly hatched chicks (Ross 308) were randomly assigned to 4 experimental groups (n=63/group), each with 6 replicate pens (n=10-11 birds /pen). The birds received daily either a control diet (CON) without access to BSFL or CON plus BSFL amounting to 10% (BSFL_10), 20% (BSFL_20) or 30% (BSFL_30) of the feed intake (fresh mass) over a period of 6 weeks (wk). Feed intake of the CON birds was the basis for the calculation of BSFL given to the other 3 groups on the next day (d). CON diet was provided to all birds in age-specific formulations, corresponding to a starter (d 0-14), grower (d 15-28) and finisher feed (d 29-42). Average DM contents of the CON starter, grower and finisher feed as well as BSFL were 89.3, 89.1, 89.2 and 31.2% respectively. CON diet and water were offered ad libitum. Pen based feed consumption was measured daily. All birds were individually weighed weekly throughout the experiment. Pen based average body weight (BW), feed and associated nutrient and energy intakes, and energy and protein conversion ratios were calculated. Data were analyzed using MIXED procedure (PROC MIXED) of SAS (V9.4). Least square means were separated using the Tukey test ($P<0.05$).
Results: In general, all BSFL offered to the birds were consumed within few minutes in all 3 BSFL groups. The BSFL_30 group consumed less feed than all other groups ($P=0.001$), whereas total fresh matter intake (feed + larvae) did not differ among the groups ($P=0.124$), resulting in less total dry matter intake (DMI) in BSFL_30 than in CON ($P<0.05$). The lower DMI caused by the consumption of BSFL at the highest level (30%) did not affect protein intake ($P=0.329$), but resulted in higher fat intake ($P<0.05$). Overall, the total energy intake of BSFL_30 birds was lower as compared to CON. There were no BW differences among groups until 4 wk of age ($P>0.05$). At wk 5, CON tended to have higher BW than BSFL_30 ($P=0.098$). In the end of the experiment BSFL_20 birds became heavier than BSFL_30 birds ($P<0.05$). Overall, the amount of dietary protein to gain 100 g BW (i.e. protein conversion ratio, PCR) was higher in BSFL_30 than in CON ($P<0.05$). Energy conversion ratio (MJ/100 g BW) was higher in BSFL_30 and BSFL_10 than in BSFL_20 only at wk 5. As compared to CON, the lower DMI in BSFL_30, likely due to high fat intake ($P<0.05$), did not influence energy conversion ratio ($P>0.05$). In contrast, protein was less efficiently converted into BW of BSFL_30, even if the amount of consumed protein was not different between CON and BSF_30 ($P>0.05$). These differences indicate a relative surplus supply of protein in the BSFL_30 group. There were time-dependent differences in total Ca and P intake between groups ($P<0.05$), which might further be associated with growth performance of the birds.
Conclusions: Broilers show a strong preference to consume BSLF over regular feed. Since growth performance of the chickens did not differ among CON, BSF_10 and BSF_20 groups, we conclude that BSFL can be included in broiler diet up to 20% of their feed consumption. Higher levels of BSFL consumption may hamper growth performance, likely due to lower energy intake and an impaired protein utilization efficiency.
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2.

Effects of dietary inclusion of rye for broilers on growth performance, litter quality and gut morphology

Auswirkungen eines Roggeneinsatzes im Mischfutter für Broiler auf Leistung, Einstreuqualität und Darmmorphologie

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For many decades, rye acreage has decreased, with corn, wheat and triticale becoming the more preferred alternatives. Undoubtedly, actually the interest in rye has again increased, particularly in recent years [1], and could be interesting in terms of the sustainability debate. However, the use of rye for intensive poultry production has been limited to date. This study aimed to test whether rye could replace corn or wheat and to estimate the effect of upper inclusion levels of crushed corn or squashed rye of up to 30% in broilers on growth performance, litter quality and gut morphology. **Methods:** A total of 256 broilers, Ross 308, were randomly allocated into 32 pens. From day 14 till day 42, the birds were divided into four feeding groups (eight replicates each). One finisher diet (control diet) based on wheat and soybean meal was used for the control group throughout the trial. In order to maximise the comparability of the finisher diet, two special pelleted supplementary feeds were produced. Each consisted of a pelleted supplementary feed (supplementary feed to corn (SFI), supplementary feed to rye (SFII), to which increasing amounts of crushed corn (SFI-Corn) or squashed rye (SFII-Rye) were added instead of wheat as main cereal component in the control diet. The fourth group received a mixture of 50% SFI-Corn and 50% SFII-Rye (Mixed). The level of the crushed corn or squashed rye was increased weekly (5%, 10%, 20% and 30%, respectively). Recording of body weight, litter dry matter and foot pad scoring were performed weekly, while the histopathological investigations were done at d 42 of life. The statistical analysis was performed using the Statistical Analysis System for Windows the SAS® Enterprise Guide®, version 9.3. Shapiro-Wilk test for normal distribution was performed and normally distributed data were checked for significant differences with the Ryan-Einot-Gabriel-Welsch-Test (simple Anova). The $p < 0.05$ formed the basis of statistical significance. **Results:** No significant effects were noted between experimental diets for feed and water intakes during the entire trial period (d 14-d 42). The water:feed intake ratio showed no significant differences between all the experimental groups. The growth performance level of the broilers in the present study was high (range: 2932-3038 g) and exceeded the Ross 308 performance standards except for the broilers in the mixed group (2899 g) at d 42. Interestingly, no significant effect was observed regarding the increasing percentage of crushed corn or squashed rye in diets on body weight weekly except at d 21. The body weight gain did not differ significantly between the experimental groups. However, body weight gain for the control group was ~5.65% higher than for birds in the mixed group. The control diet, however, tended to have a significantly more favourable feed conversion ratio (1.60) compared to the other treatments, whereas the feed conversion ratio was not significantly affected between groups fed SFI-Corn and SFII-Rye diets during the entire experimental period (d 14-d 42). Litter dry matter content from d 14 till d 41 was not significantly affected by the different experimental diets. The control group, however, had the driest litter (47.7% dry matter) at 41 d of fattening. No significant differences in the pododermatitis scores between the groups were noted (< score 2). Birds fed SFI-Corn showed a significant lower ileal villus height (425 µm) compared to other groups. No significant differences were observed between the experimental groups for the morphological caecal parameters. However, birds fed SFI-Corn had the highest ceacal villus height (195 µm) vs. the lowest ceacal villus height (178 µm) for those fed SFII-Rye. **Conclusion:** Including rye in broiler diets beginning with 5% in the third week of life, increasing to 30% of rye in the last weeks of the rearing period did not lead to significantly effects on feed intake, growth performance, litter quality and foot pad health. Thus, there are possibilities of using rye in higher dietary levels if it is fed in squashed form to pellet diets and at older age.

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3.

Fermentation of rapeseed cake with or without enzymes for broiler nutrition

Fermentation von Rapskuchen mit oder ohne Enzyme für die Broilerernährung

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The present study investigated the impacts of spontaneous and enzymatic fermentation of rapeseed cake (RSC) on nutritional quality of RSC. It also evaluated the effects of partial substitution of soybean meal (SBM) in broiler diets with processed RSC on growth performance and nutrient digestibility.

Methods: RSC was fermented without (spontaneous) and with a mixture of phytase (1609 FYT/kg RSC, RONOZYME® HiPhos, DSM, Switzerland), pectinase and β-glucanase (enzymatic; 45 FBG/kg RSC of β-glucanase, RONOZYME® VP, DSM, Switzerland). A standard corn-wheat-SBM diet was used as control diet (CON). Three experimental diets were produced using native (NR), spontaneously (SFR) or enzymatically fermented (EFR) RSC as substitutes for SBM at 150 g/kg inclusion level. A fifth diet (NRE) was produced using NR diet and in-feed inclusion of the same amount of enzymes present in EFR diet. The experimental diets were formulated to be isocaloric and isonitrogenous. The grower (d 22-35) diets contained 3 g titanium dioxide per kg feed (Sigma Aldrich, St. Louis, MO) as an indigestible marker to allow for determination of the apparent ileal digestibility (AID) of nutrients. The trial lasted 35 d. Growth performance variables were recorded weekly. Data were subjected to ANOVA using the GLM procedure.

Results: Enzymatic fermentation drastically reduced phytate in RSC (8.35 vs. 0.25 mg/g DM fat-free) while spontaneous fermentation of RSC had no impact on phytate content. Spontaneous and enzymatic fermentations reduced insoluble (11.29 and 12.80 vs. 14.48 % DM- fat-free) and total NSP (13.32 and 14.70 vs. 16.40 % DM fat-free) content of RSC. However, spontaneously fermented RSC displayed slightly lower soluble NSP and enzymatically fermented RSC showed slightly higher soluble NSP compared with native RSC (1.91 and 2.02 vs. 1.93 % DM fat-free). At the end of the starter period (d 21) and entire experiment (d 35), feed conversion ratio (FCR) of broilers received NRE diet (1.40 and 1.48, respectively) was lower compared with those fed NR (1.51 and 1.56, respectively) or CON (1.55 and 1.55, respectively) diets ($P \leq 0.05$). At the end of the starter period, birds fed EFR diet (1.41) showed better FCR than those fed CON and NR diets, while at the end of the experiment, birds in EFR group (1.49) displayed similar FCR to birds in CON group (1.55) but better FCR compared with those in NR (1.56) group ($P \leq 0.05$). There were no differences among FCR of the experimental groups during the growing period ($P > 0.05$). The experimental diets had no effect on body weight gain and feed intake of broilers during different experimental periods ($P > 0.05$). The AID of Gly in broilers fed CON diet (85.4 %) was lower than those fed SFR and EFR diets (87.7 % and 88.8 %, respectively), while the AID of Gly in broilers fed diet containing enzymatically fermented RSC was higher than those received NR (85.8 %) and NRE (86.0 %) diets ($P \leq 0.05$). The AID of Cys in CON (80.4 %) and native RSC (81.2 %) groups was lower than in SFR (83.4 %) and NRE (83.5 %) groups ($P \leq 0.05$). Birds fed diets containing enzymes (EFR and NRE) showed higher AID of P (61.4 and 59.6 %, respectively) compared with those received CON, NR and SFR (52.2, 52.3 and 48.9 %, respectively) diets ($P \leq 0.05$). NR diet displayed the lowest (45.0 %) AID of Ca ($P \leq 0.05$). The AID of Ca in EFR (53.7 %) and CON (53.3 %) groups was higher than NR group ($P \leq 0.05$) but similar to SFR and NRE groups ($P > 0.05$). NRE diet showed higher AID of Ca (55.5 %) compared with SFR diet ($P \leq 0.05$).

Conclusion: In conclusion, enzymatic fermentation of RSC effectively reduced anti-nutrients. Inclusion of 150 g/kg native or fermented RSC in broiler diets as substitutes for SBM had no negative impact on digestibility of the nutrients and growth performance of broilers. Furthermore, inclusion of enzymatically fermented RSC in broiler diets and supplementation of diets containing native RSC with an enzyme mixture could improve the AID of some of the nutrients and led to better feed efficiency for these diets.

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4.

Amino acid digestibility and metabolisable energy of spring and winter faba beans grown on two sites in caecectomised laying hens

Aminosäurenverdaulichkeit und Umsetzbare Energie von an zwei Standorten angebauten Sommer- und Winterackerbohnen bei caecectomierten Legehennen

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Level and variation of amino acid digestibility (AAD) and nitrogen-corrected metabolisable energy (MEN) of faba beans in laying hens are unknown. Differences in precaecal AAD and MEN between spring and winter faba beans have been reported for broiler chickens [1]. Environmental and management conditions during cultivation might contribute to variation in AAD and MEN. This study examined the variation in AAD and MEN in spring and winter faba beans. Relationships between chemical constituents of the faba beans and AAD or MEN were studied. The effect of dehulling on AA digestibility and MEN was also investigated.

Methods: Sixteen non-dehulled faba bean variants comprising four spring and four winter genotypes, each harvested in Nimtitz and Göttingen, were investigated. These locations differed in precipitation as well as total nitrogen and available phosphorus in the soil. Genotypes comprised breeds with widely varying vicine/convicine concentrations. One of the low vicine/convicine genotypes grown in Nimtitz was additionally tested after being mechanically dehulled. The maize/soybean meal-based experimental diets consisted of 250 g/kg of either one faba bean variant or maize starch. Diets met or exceeded the nutrient recommendations of the GfE [2]. Pelleted diets were fed to ten caecectomised laying hens in a row-column design so that five replicates were obtained from each diet. In each experimental period, hens received 115 g/d of the respective diet for eight days while being housed individually in metabolism units. Excreta were quantitatively collected during the last four days of each period. AAD excluding basal endogenous losses was determined using the regression approach.

Results: Higher concentrations of crude protein, phosphorus, and phytate and lower starch concentrations were determined for the winter genotypes grown in Nimtitz compared to the other faba bean variants. Dehulling reduced concentrations of tannins and crude fibre, and increased starch concentrations. Levels of AAD differed widely among AA with highest values determined for Arg (90-93%) and lowest for Cys (-12-65%). These AA also featured the lowest and highest range in digestibility, respectively. Digestibility of further important AA was 63-82% for Met, 83-90% for Lys, and 66-79% for Thr. MEN ranged between 10.3 and 12.3 MJ/kg dry matter among the non-dehulled faba bean variants. The interaction between cultivation site and season type was significant ($P \leq 0.043$) for the digestibility of Cys, Glu/Gln, Phe, Pro, Tyr, and Val, and for MEN. Among the variants grown in Nimtitz, digestibility of these AA was by 5 - 12 %-units and MEN was by 0.8 MJ/kg dry matter higher in the spring compared to the winter variants ($P \leq 0.042$) while there was no difference between the spring and winter variants grown in Göttingen ($P \geq 0.138$). Serine digestibility in the spring variants was by 3 %-units higher than in the winter variants ($P = 0.043$). Phytate concentrations were negatively correlated ($P < 0.050$) with the digestibility of five AA. Tannins or vicine/convicine were not significantly correlated with AAD and MEN. Dehulling increased MEN by 1.7 MJ/kg dry matter ($P = 0.003$) and raised the digestibility of Glu/Gln, His, and Pro ($P < 0.050$).

Conclusions: Variation in AAD was partly explained by the phytate concentration of faba beans. This may have been caused by features of the two cultivation sites, like available phosphorus in the soil or precipitation. Tannin and vicine/convicine concentrations were no relevant causes for variation in AAD and MEN. Dehulling increased MEN and the digestibility of some AA.

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5.

Can the larvae of the black soldier fly replace soybean in the diet of broilers?

Können die Larven der Schwarzen Waffenfliege in der Ernährung von Broilern Soja ersetzen?

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Soybean meal and oil are important protein and energy sources in poultry nutrition, but due to the high amount of imported soybeans to Europe, and associated environmental impacts, its use is discussed (1). One promising alternative to soybean-based feeds could be insects. Particularly the Black Soldier Fly (Hermetia illucens) larvae (BSFL) are rich in limiting amino acids (AS), energy and grow on a variety of organic wastes and side streams (2). By now, their use as feed ingredient for poultry is not yet permitted in Switzerland and the EU. However, as some selected insect species are authorised for pets, aquaculture and human consumption, it is expected to change soon. It would be most sustainable if the the entire larvae would be used in poultry feed. However, so far the feeding value of BSFL-based feeds has mostly been determined with partially defatted meal, and differences between BSFL batches reared under different conditions (e.g. substrates, rearing conditions) have rarely been studied. For this purpose, the defatted BSFL meal and fat from two origins were included in the diet of broilers instead of soybean, and the influence on growth and slaughter performance as well as meat quality were investigated. **Methods:** Eighty Hubbard S757 broilers were kept in pairs, randomly distributed to five groups and fattened for 9 weeks with one of five diets each. A positive control diet (SS) was based on soybean meal and oil and designed to meet the nutritional recommendations for this broiler type. To determine whether the protein value of the insect material was really comparable to that of soybean meal, the other diets contained only about 170 instead of the 210g crude protein (CP)/kg DM recommended, thus also inducing a calculated deficiency of limiting AS. The latter was also applied to diet SS-, which was also based on soybean meal and oil. The insect-based diets consisted of defatted BSFL meals and fats from two different origins. Diet AA- and BB- contained larval meal and fat from origin A and B, respectively (actually only the partially defatted meal B was used for BB- due to high residual fat amounts), and diet AB- was based on BSFL meal A and fat B. The experimental diets were fed from week 3 to the end of week 9. In this time, feed intake was determined daily and body weight weekly. Various traits of carcass and meat quality were determined after slaughter. Data were evaluated by Mixed Procedure and post-hoc Tukey contrasts (SAS 9.4). **Results:** The analyzed CP contents were very similar in the insect-based diets and SS-. Contents of lysine in these four diets were 10 to 26% below recommended levels. Those of the Sulphur containing AS of diets AA-, AB- and BB- were 24 to 29% below recommended levels and those of SS- even 9 to 39%. Despite this, the average daily gains (g/day) of the birds in groups AA- (27.5) and AB- (26.4) did not differ from the control group (27.1). The birds of groups SS- (20.4) and BB- (19.9) grew slower than the other groups, and also feed intake was decreased (both $p<0.05$). Feed conversion efficiency with both diets was impaired compared to diet SS ($p<0.05$). The carcass weights of the groups SS- and BB- (0.8 kg) were lower ($p<0.05$) than those from groups SS, AA- and AB- (highest with 1.1kg in SS). The breast meat percentages were lower ($p<0.05$) with diets SS- and BB- compared to the other groups. Cooking loss of the breast meat differed ($p<0.05$) between SS (14 %) and BB- (17 %), and shear force of the meat of group BB- was highest ($p<0.05$) with 15.2 N. **Conclusion:** The results indicate that, depending on the origin, feeds based on BSFL may have a comparable nutrient value with soybean in broilers and thus are suitable to fully replace soybean meal and oil. The currently recommended supply with limiting AS for organic broilers may be set too high.

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6.

Effect of Hermetia illucens larvaeprotein meal and fat on zootechnical performance of broilers

Einfluss der Fütterung von Proteinmehl und Fett aus Hermetia illucens auf die zootechnische Leistung von Masthühnern

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The high demand for protein feeds in livestock production is mainly covered by imports of protein feedstuffs from South America. Against the background of this strong import dependency, investigations of alternative protein sources gain special importance. Insects constitute a valuable feedstuff for animal nutrition, protein content of which can be increased by means of defatting in order to generate a protein meal (1). Moreover, separation of protein and fat may enable a more balanced and precise diet formulation. Therefore, the present experiment aimed to investigate the suitability of Hermetia illucens (HI) larvae protein meal and fat for broiler nutrition. We hypothesized that the replacement of soybean meal (SBM) and oil with HI larvae meal and fat will not affect broiler animal performance. **Methods:** In total, 432 male Ross 308 1-day old broiler chickens were randomly assigned to one of six dietary treatments and allotted to 36 pens with 12 animals per pen, resulting in six replicates per treatment. The experiment was designed as 2 × 3 factorial design. Thus, the following treatment groups were created: 1) control diet based on SBM and soy oil (CON), 2) diet consisting of SBM and 50% soy oil and 50% HI larvae oil (SBM-50HI), 3) diet with SBM and 100% HI larvae oil (SBM-100HI), 4) diet where 15% of SBM crude protein was substituted with HI larvae meal and soy oil (15HI-0HI), 5) diet with HI larvae meal and 50% soy oil and 50% HI larvae oil (15HI-50HI) and 6) diet with HI larvae meal and 100% HI larvae oil (15HI-100HI). A three-phase feeding program was used: starter (1-14 d), grower (15-28 d), finisher (29-35 d). Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion rate (FCR) were determined penwise on days 1, 14 and 28, as well as individually on day 35. Statistics were performed using PROC MIXED of SAS v 9.4 with protein and fat as fixed effects, and pen as random effect. **Results:** In the starter phase, protein source significantly affected BW and ADG with on average 11 g higher BW for HI larvae meal groups compared to SBM groups. Moreover, an interaction of protein and fat was observed for BW with SBM-100HI being lower than CON, 15HI-0HI and 15HI-100HI. Likewise, ADG for SBM-100HI was lower than 15HI-0HI and 15HI-100HI. In the grower phase neither protein nor fat source affected zootechnical performance characteristics. However, in the finisher phase HI larvae meal increased ADFI by 9 g, which nevertheless did not result in a higher BW. **Conclusion:** The present experiment shows that partial replacement of SBM with HI larvae meal and total substitution of soy oil with HI larvae oil has no negative impact on animal performance. In fact, using HI larvae meal as protein source even improved growth performance of the birds during the starter phase.

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7.

Effect of heat treatment of different soybean varieties on amino acid digestibility and gut microbiota in broiler chickens

Effekt unterschiedlicher Hitzebehandlungen von zwei Sojabohnensorten auf Aminosäurenverdaulichkeit und Darmmikrobiom in Broiler

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Antinutritive factors occurring in soybeans are well known to affect digestibility of livestock feed. Optimal amino acid digestibility requires a heating process to soybean products before feeding to monogastric livestock. Besides desired elimination of antinutritive factors unintended heat damages can occur, which might affect animal´s performance negatively. A high fraction of the heat induced changes is due to the maillard reaction, where amino groups are reacting with reducing sugar molecules to form typical products. As a result, amino acids can get unavailable for animal nutrition [1][2]. Maillard reaction products like hydroxymethylfurfural or carboxymethyllysine may interact with the gut microbiota, thus being crucial for animal health and performance [3]. This study investigated two selected soybean varieties, treated at two different heating intensities, for their ileal amino acid digestibility and on its effect on gut microbiota.

Methods: Soybean varieties were chosen by their response to heat on the basis of protein solubility in 0.2% potassium hydroxide. The variety with high loss of protein solubility during a heating process was defined as heat susceptible, whereas little loss of protein solubility was associated with low heat susceptibility. Partially defatted soybeans were exposed to two different heating intensities using an autoclave. Soybeans were either heated 20 minutes at 110°C (plus 20 minutes for heating-up and cooling-down) or treated at 120°C for 20 minutes (plus 45 for heating-up and cooling-down). After heat treatment the soybean variety considered as more heat susceptible had protein solublity of 78% and 48%, respectively. The soybean variety with low heat susceptibilty had protein solubility of 81% and 61%. Soybeans were integrated into a broiler diet at a rate of 30% to reach the nutrient recommendation of Aviagen 2014. The feeding trial (36 fattening days) was carried out with 336 one-day-old chickens (Ross 308). Animals were distributed equally in four treatments among 24 pens. Six and four animals per pen were used to collect digesta of ileum and caecum, respectively. Ileal digesta of six animals per pen were pooled for amino acid analysis and four individual digesta samples per pen were used for microbiota analysis. Amino acid digestibility was determined as disappearance of amino acids between feed intake and content of the lower two-thirds of ileum, using titanium dioxide as indigestible marker.Microbiota of ileum and caecum was characterized via 16S rRNA amplicon sequencing. Data was subjected to a 2-factorial ANOVA using the factors "variety", "heating temperature" and "interaction".

Results: Intensity of heat treatment exerted some effect on the apparent prececal amino acid digestibility. Digestibility of asparagine ($p<0.01$) as well as of cysteine, lysine, histidine, glycine, proline and glutamine ($p<0.1$) was reduced in the soybeans treated at high temperatures compared to those prepared at low temperatures. No significant differences for variety and no interactions were observed. Most abundant bacterial class in the ileum were bacilli, whereas the majority of caecum bacteria belonged to clostridia. No significant differences in alpha diversity was observed between treatments, although some significant differences ($p<0.05$) in abundance of bacterial families were detected. In the ileum, Clostridiaceae was more frequent in broilers fed the heat susceptible variety compared to the less heat susceptible one, and abundance of Erysipelotrichaceae was increased with high temperature treated beans compared to low temperature treated beans ($p<0.05$). In the caecum Erysipelotrichaceae, Lachnospiraceae and Streptococcaceae were more abundant when feeding the low susceptible variety compared to the heat susceptible variety ($p<0.05$). Presence of Peptostreptococcaceae was elevated when feeding the high temperature treated soybeans compared to the low temperature treated soybeans ($p<0.05$).

Conclusions: An intensive heat treatment reduced the apparent prececal digestibility of the heat labile amino acids in broiler chickens. The application of different soybean varieties as well as different heating intensities led to changes in the composition of gut microbiota in ileum and caecum.

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8.

Impact of selected feedstuffs rich in polyphenols and dietary fibre on performance and digestibility of nutrients in broilers

Einfluss verschiedener Futtermittel mit hohem Gehalt an sekundären Pflanzenstoffen und Faser auf zootechnische Leistung und Nährstoffverdaulichkeit beim Broiler

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Polyphenols are substances of complex chemical structure. These secondary plant metabolites show antimicrobial, antioxidative and immunomodulating properties (1). In plants, polyphenols are contained together with other plant metabolites and with varying levels and sources (soluble/insoluble) of dietary fibre (DF) (2), exacerbating the argumentation on the mode of action of these components. Depending on various factors, these compounds are known to additionally affect poultry performance and nutrient digestibility (3). We investigated the impact of different plant sources with varying levels of polyphenols and DF in broiler diets on the apparent ileal digestibility (AID), apparent total tract digestibility (ATTD) of nutrients and the apparent metabolizable energy (AMEN). We hypothesized that there is a negative correlation of total phenols (TP), condensed tannins (CT) and DF content of feed with the digestibility of nutrients. While high contents of polyphenols primarily impair protein digestibility, higher DF content affects digestibility of all nutrients to a similar extent.

Methods: 432 one day old unsexed broiler chicks (Ross 308) were equally distributed to 6 feeding groups (6 replicates) consisting of a control (basal feed, CON) and 5 feeding groups with 1.5% of alfalfa meal (ALF, TP: 11.5 g gallic acid equivalents (GAE)/100g DM, 543 g total DF (TDF)/kg DM), red clover (RCL, TP: 22.4 g GAE/100g DM, 429 g TDF/kg DM), grape skin meal (GSK, TP: 54.7 g GAE/100g DM, 490 g TDF/kg DM), grape seed meal (GSM, TP: 88.7 g GAE/100g DM, 650 g TDF/kg DM) or aronia pomace (ARP, TP: 72.1 g GAE/100g DM, 652 g TDF/kg DM) added on top. A starter (d1-d14; 12.4 MJ AMEN/kg, 220 g CP/kg), grower (d15-d28; 12.8 MJ AMEN/kg, 210 g CP/kg) and a finisher diet (d29-d38; 12.9 MJ AMEN/kg, 190 g CP/kg, 0.3% TiO_2 as inert marker) were provided ad libitum in ground form. Excreta was collected penwise on days 33-35, ileal digesta of 6 representative broilers was pooled penwise during slaughter. Apparent ileal digestibility and ATTD of nutrients were calculated. ANOVA was computed using GLM of SAS 9.4. with Tukey as a post hoc test and significance level at $p<0.05$ and trends at $p<0.10$.

Results: Experimental feedstuffs did not affect overall performance of broilers; however, GSM and ARP resulted in lower ADG compared to CON in the starter phase and there was a trend for higher FCR in ARP compared to CON ($p<0.10$) in the grower phase. Concerning carcass characteristics, GSM showed the lowest weight of chilled carcass ($p<0.10$) and offered the lowest abdominal fat content. While AID of dry matter (DM) and organic matter (OM) was mostly impaired by GSK and GSE compared to CON, ATTD of DM, OM, ash and gross energy (GE) were affected by RCL, GSK and GSE. Alfalfa supplementation improved AID of DM and starch compared to GSK, whereas RCL improved AID of CP compared to ARP. No effect was observed in ATTD of CP and AMEN. Total phenols and CT showed a negative correlation with AID of ash, whereas NDF was negatively correlated with AID of DM, OM and ash. Concerning ATTD, fibre parameters (esp. NDF, ADF, TDF (IDF+SDF)) negatively correlated with ATTD of DM, OM, ash and GE.

Conclusions: Despite insignificant effects were observed on overall performance, grape by-products showed the strongest negative effect on carcass characteristics and digestibility, indicating a too high DF content in the respective diets. Unlike our expectations, TP and CT content mostly affected mineral instead of protein digestibility, suggesting complexation of minerals. Under the dietary conditions tested, alfalfa is the most promising source for broiler diets, indicating that polyphenol sources in broiler feeding may be of less relevance.

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9.

Digestibility of two batches of the same lab mouse diet in pelleted and extruded form in mice

Verdaulichkeit zweier Chargen derselben Diät für Labormäuse in pelletierter und extrudierter Form

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Introduction: In experiments with laboratory animals, standardisation is of the utmost importance. Diet composition and processing may have a significant impact on feed utilisation and digestibility (1,2). Commercial maintenance diets for lab mice are often available in several confections (pellets, extrudate, powder, or paste) but marketed as exchangeable when used.

The present study aimed to demonstrate the impact of confection on digestibility in lab mice.

Methods: We used 8-week-old C57BL/6 in two trials. In trial 1, eleven mice per group were fed a commercial, fortified lab animal maintenance diet in pelleted (P1) or extruded (E1) form. The degree of starch gelatinization (measured according to VDLUFA standard method III 7.2.6) of 15.2% and 56.7% in diet P1 and E1, respectively. The content of nitrogen-free extracts (NfE) was 57 and 57% DM. After one week of adaptation to the respective diets, the total amount of faeces per cage (2-3 mice) was collected for 15 days. In trial 2, two groups (n=16/group) were used in a similar set-up. They were fed new batches of the same diets (P2, E2). Starch gelatinisation was 17 and 50%, NfE content 65 and 55% DM. Diets and faeces of both trials were analysed for macronutrients to calculate the apparent digestibility (aD) of gross energy (GE), crude protein (CP), ether extracts (EE), and the carbohydrate/fibre fraction (N-free extracts + crude fibre; CH+F). Groups were compared via one way ANOVA (SigmaPlot software; significance level: $p<0.05$).

Results: There were significant differences between the aD of DM (P1: 70.1%a, E1: 77.2%b, P2: 81.3%c, E2: 77.6%b; means sharing the same superscript letter do not differ significantly; $p<0.001$), GE (P1: 74.7%a, E1: 81.0%b, P2: 84.4%c, E2: 81.9%d, $p<0.005$) and the CH+F fraction (P1: 69.2%a, E1: 79.1%b, P2: 85.1%c, E2: 81.7%b, $p<0.001$). There was no significant difference between aD(CP) in trial 1, only in trial 2 (P1: 82.1%a, E1: 81.7%a, P2: 83.8%b, E2: 85.1%c, $p<0.001$). aD(EE) did not show a distinct pattern (P1: 91.2%a, E1: 92.0%a,b, P2: 93.2%b, E2: 93.4%b, $p<0.05$). In retrospect, we analysed the starch content of all diets and found that diet P2 contained ~50% more starch (49% DM) than the other three diets (30-32% DM), with corresponding differences in total dietary fibre content.

Conclusion: The results demonstrate that the total starch content as well as the impact of processing on starch gelatinisation lead to differences in diet digestibility in laboratory mice. The percentage of metabolisable energy from starch differs considerably between batches of pelleted diets as well as between confections. This may affect gastrointestinal and intermediary metabolism. Information on these parameters should be considered when planning a lab animal experiment. Regarding standardisation, there seem to be inconsistencies between batches of the same pelleted diet, compromising the reproduction of results.

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10.

Effect of bakery products on feed efficiency and microbial abundances in feces of fattening pigs

Einfluss von Bäckerei-Nebenprodukten auf die Futtereffizienz und mikrobielle Abundanzen im Kot beim Mastschwein

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Surplus produced bakery products (BP), including bread, buns and pastries, have become attractive as alternative feedstuffs to substitute part of the cereals in pig diets. In contrast to cereals, BP generally comprise higher amounts of fat, sugar and sodium chloride, which may have consequences for the animal product quality and health. Especially high amounts of mono- and polyunsaturated fatty acids in bakery products may rise intestinal oxidative stress levels and impact the gut microbiota and carcass fat composition. However, scientific-based knowledge on performance, gut health and carcass traits is still rare; partly due to the little predictable composition of BP that are daily returned from the stores. The present pilot study aimed to evaluate the effect of a dietary inclusion of 30% bakery products in diets for fattening pigs on feed intake, growth performance and feed efficiency as well as on the fecal microbiota. We hypothesized that the BP may increase pig's feed intake, reduce animal's feed efficiency and modulate microbial numbers in feces.

Methods: A total of 36 growing pigs (Large White × Piétrain; starting body weight 31.3±1.0kg) were randomly allocated to the control (n=18; n=9/sex) or BP diet (n=18; n=9/sex). Diets were based on wheat, corn, barley and soybean meal, and contained similar amounts of crude protein and metabolizable energy. BP were included at a level of 30% and replaced mainly wheat and corn. Diets were fed for 11 weeks using Feed Intake Recording Equipment feeders, with bi-weekly body weight measurements. DNA from feces collected in week 7 was extracted for microbial quantification using real-time PCR. Feed efficiency metrics (residual feed intake (RFI), residual body weight gain (RBG), residual feed intake and body weight gain (RIG) as well as feed conversion ratio (FCR)) were computed using regression analysis in SAS. Data of feed intake, growth, feed efficiency and microbes were subjected to ANOVA in SAS.

Results: BP diet contained 21 g/kg more fat than the control diet. Fatty acid profiles showed a 15.0%-decreased proportion of linoleic acid in the BP compared to the control diet, whereas caprylic, lauric and myristoleic acid were only detectable in the BP diet. Barrows ate more and were heavier than females during the fattening period ($P<0.05$). However, barrows and females had similar feed intake when fed the BP diet but not when fed the control diets in week 1 ($P<0.05$), 2 and 3 ($P<0.1$) as indicated by the sex × diet interaction. Moreover, barrows and females tended ($P<0.1$) to eat more when fed the BP compared to the control diet in week 10. The sex × diet interaction showed that both sexes had similar body weight when fed the BP diet, whereas barrows were heavier than females when fed the control diet in week 11 ($P<0.05$). The FCR was lower in females than in barrows ($P<0.05$), but when expressing the feed efficiency on the basis of RFI, RBG or RIG no differences between sexes were observed. Irrespective of the feed efficiency metrics used, dietary inclusion of BP did not affect pig's feed efficiency. With regard to the microbial quantification, the BP diet decreased the abundance of fungi and yeasts in feces compared to the control diet ($P<0.05$). This finding may be related to caprylic, lauric and myristoleic acid, which show anti-fungal properties, and were present in the BP but not in the control diet.

Conclusion: Present results showed that the substitution of cereals in diets for fattening pigs by BP, even at a dietary level of 30%, did not impact the feed efficiency when determined for the entire fattening period. Nevertheless, present feed intake data indicated that bakery products may be differently 'accepted' by barrows and female pigs which may be related to the taste of the feed or intestinal signaling (e.g. gut incretins). Results further showed the importance to characterize the fatty acid composition of the BP to estimate consequences for carcass quality and potential anti-microbial effects in the gut.

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11.

Effect of feeding young grass-clover-silage in the diet of fattening pigs on fattening performance and carcass quality

Effekt der Fütterung von junger Rotkleegrassilage in der Ration von Mastschweinen auf Mast- und Schlachtleistung

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Due to its high crude protein content and favorable amino acid profile, red clover has a potential in pig feeding. Furthermore, red clover can be a material to manipulate. Since the crude protein content decreases and the crude fiber content increases during plant maturation, early cut small-grain legumes have a better feed value for pigs. The aim of our study was to evaluate the effect of a restricted feeding of grass-clover silage in the diet of fattening pigs under the conditions of organic farming.

Methods: The experiment took place at the experimental farm of the Thünen Institute of Organic Farming in Northern Germany. Grass-clover silage for feeding trials was cut in May before pod formation and pressed at the same day. It was analyzed for DM content and for grass:clover:others ratio as well as for Weende crude nutrients and amino acid contents (VDLUFA methods). Two identical trials were conducted in 2019 and 2020. The fattening pigs were born on the farm ((Norwegian land race*large white)/Pietrain) and raised according to the standard protocol of the farm (47d suckling period followed by group housing). All animals had access to grass-clover silage until pre-fattening period (28 kg BM). In each trial a total of 80 pigs was separated into 2 groups with 4 replicates each. In each trial, one control group (Straw) was fed standard fattening diets (30-75 kg BM: 12.9 MJ ME, 167.0 g CP, 9.9 g Lys, 75-123 kg BM: 12.5 MJ ME, 141.0 g CP, 7.1 g Lys per kg feed with 88% DM) without access to silage (feeders filled with straw). The other group (Silage) was fed diets, that were lowered in energy, protein, and lysine content (30-75 kg LM: 12.6 MJ ME, 156.5 g XP, 9.4 g Lys, 75-123 kg LM: 12.0 MJ ME, 123.0 g CP, 6.1 g Lys per kg feed with 88% DM) with access to an increasing amount of grass-clover silage (30-50 kg LM: 0.5 kg/d, 50-75 kg LM: 1.0 kg/d, 75-123 kg LM: 1.5 kg/d). Trial diets were formulated to contain less to no oil cakes (especially soy) and more local feed. Until 50 kg BM pigs were fed semi ad libitum. From 50-75 kg BM the feed intake was restricted to 2.2-2.5 kg/d and from 75-123 kg to 2.7-2.9 kg/d. The pigs were weighed weekly and slaughtered upon reaching 119 kg body mass in order to maintain a mean slaughter body mass of 121 kg. Dressing percentage, lean meat content, and pH value after 60 minutes were observed at the slaughter house. A general linear mixed effects model was used in R (package lmerTest, R stat version 4.0.2) to compare the feeding groups. Group was used as fixed factor, trial run, sex, pen and sow were used as random factors.

Results: The silage for the feeding trial contained 187 g crude protein 9.5 g lysine and 2.6 g methionine per kg DM. It consisted of about 49% ryegrass, 49% red clover, and 2% others. The pigs fed with a diet adapted for grass-clover silage feeding gained the same weight as the control group (Table 1).

Table 1: Fattening performance and carcass characteristics

	Straw	Silage		p value
	Mean	Mean	SE	
Daily weight gain (g)	855	855	51.4	1.00
Daily weight gain pre-fattening (g)	728	763	101.0	0.02
Daily weight gain medium fattening (g)	884	896	67.3	0.56
Daily weight gain final fattening (g)	929	888	25.5	0.03
Dressing percentage (%)	79.5	79.1	0.37	0.18
Lean meat content (%)	59.5	59.5	1.31	0.97
pH 60	6.60	6.65	0.08	0.29
Body mass at slaughter (kg)	121	122	0.60	0.63
Age at slaughter (d)	183	183	7.2	0.80

Pigs that were fed with silage instead of straw, gained more weight in pre-fattening and less in final fattening. However, carcass characteristics did not differ between the two feeding groups.

Conclusions: The use of young grass-clover silage instead of straw as a roughage in organic farming has the potential to be used as high quality fiber and protein feed to improve the regional production of pigs.

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12.

The effects of hay harvested with an impeller mowing conditioner on chewing behavior, feed intake, and performance of organic dairy cows as well as on apparent total tract digestibility of nutrients.

Effekte eines Zinken-Mähaufbereiters bei der Heuwerbung auf das Kauverhalten, die Futteraufnahme und die Leistung von Milchkühen sowie die scheinbare Nährstoffverdaulichkeit unter ökologischen Produktionsbedingungen.

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Impeller mowing conditioners are often used for harvest of barn-dried hay to ensure rapid wilting of forages and to reduce respiration losses. On the other hand, the mechanical stress caused by the conditioner could potentially affect the texture of forage and thus the physical effectiveness of the fiber. This could affect cows' mastication efforts and the passage rate of digesta in the rumen. In primarily forage-based feeding systems, commonly practiced in organic dairying, such effects may be of greater relevance but have not been investigated. Therefore, the aim of this experiment was to investigate the effects of barn-dried hay harvested with the use of an impeller conditioner under ceteris paribus conditions on chewing behavior, feed intake, and milk yield of cows as well as on apparent total tract digestibility (ATTD) of nutrients. **Methods:** Half of the acreage of a grass-dominated sward (2nd regrowth) was cut either with (conditioned hay) or without the use of an impeller mowing conditioner (control hay). After cutting, forages were treated equally on the field with regard to tedding and wilting times (48 ± 2 h). Forages were harvested at a dry matter (DM) content of approximately 82% by a loading wagon and barn-dried at ambient air temperature using a dehumidifier. Prior to the beginning of the feeding trial, 19 lactating Holstein cows were divided into 2 homogenous feeding groups. Groups received either the control or the conditioned hay over a period of 35 days and were also fed a fixed amount of concentrate (3.6 kg DM/d) during the trial. The concentrate mixture consisted of wheat bran, sugar beet pulp, soybean cake, maize middlings, molasses, and a vitamin and mineral premix in a 25:25:25:18:3:4 ratio (DM basis). The control and conditioned hay contained 124 and 133 g crude protein (CP), 516 and 504 g neutral detergent fiber, 197 and 175 g hemicellulose, 112 and 100 g water-soluble carbohydrates (WSC), and 5.67 and 5.54 MJ NEL/kg DM, respectively. Data collection period started after a 14-day adaptation period. Chewing behavior was measured on 7 consecutive days using RumiWatch halters, DMI and milk yield were recorded over a period of 21 days. For the determination of ATTD 9 fecal samples were taken at 8-h intervals from each cow. The statistical model (proc mixed, SAS 9.4) included fixed effects (day and treatment), random effects (cow nested within the group), and covariables derived from a preceding baseline data recording period. **Results**: Cows' eating and ruminating times were not affected by the treatment. Daily forage intake (18.4 ± 0.29 kg DM) and energy-corrected milk yield (29.4 ± 0.97 kg) also remained constant. Cows on the conditioned hay ingested lower amounts of WSC (- 190 g/d; $P < 0,01$) but at the same time they had a higher CP intake (+ 210 g/d; $P < 0.01$). This resulted in a higher milk urea content (233 vs 179 mg/L; $P < 0.01$) and negatively affected N use efficiency (28.3 vs 30.5%; $P = 0.01$). The ATTD of nutrients remained constant but pH in feces was 0.12 points higher ($P = 0.05$) in the group receiving the conditioned hay (pH 7.56) probably reflecting changes in the hindgut fermentation profile. **Conclusions**: From the results observed in this study we concluded that the conditioned hay was more prone to respiration losses on the field as compared to the control hay. The losses were mainly related to easily fermentable carbohydrates which in turn elevated indirectly the content of CP. The similar chewing times observed in both groups indicate that the physical effectiveness of fiber was not affected by the additional mechanical stress of the conditioner. Based on the similar feed intake and milk production level observed in both groups, it can be concluded that this technique did not improve forage utilization under the current feeding conditions. As this is the first study in this field further research should investigate the effects on cows' responses when using shorter wilting times of forages.

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13.

Effect of feeding bakery by-products in replacement of cereal grains on milk fatty acid composition in dairy cows

Einfluss der Fütterung von Backwaren als Ersatz für Getreide auf die Milchfettzusammensetzung bei Milchkühen

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Bakery by-products (BBP) could become alternative energy feed sources for dairy cows. Recent work has shown that feeding BBP up to 30% of diet DM in replacement of cereal grains has no detrimental effects on ruminal pH and fermentation as well as fiber degradability (1) and helps to increase dry matter intake (DMI) and milk yield (2). Substituting grains with BBP shifts the energy sources of rations from starch and fiber to more fat and sugars in the diet (1, 2). Moreover, BBP is relatively high in saturated FA (SFA) and monounsaturated FA (MUFA) as opposed to common feed sources of ruminants that are rich in polyunsaturated FA (PUFA) (1). Therefore, we expected that feeding BBP would affect milk fat synthesis due to the shift in the amount and type of substrates. The present study investigated the composition of milk FA in dairy cows fed graded amounts of BBP as a substitute for cereal grains in the diets. **Methods:** Twenty-four Simmental cows in their mid-lactation (150 ± 22 days in milk) with the average parity of 2.6 ± 1.4 (mean ± SD) were fed a 50% concentrate diet (DM basis) for 7 d (i.e., baseline). The baseline diet contained grass silage and corn silage (1:1 on DM basis) as the forage source and wheat and triticale were the starchy grain sources accounting for 30% of diet DM. Thereafter the cows were blocked by days in milk, parity, DMI, and milk yield and randomly assigned to 3 test diets consisting of CON, 15BBP, and 30BBP. Cows in the CON group continued with the baseline diet, whereas the 15BBP cows received a diet containing 15% BBP in replacement of wheat and triticale and 30BBP cows received 30% BBP and no cereal grains. All diets had the same forage source and the forage to concentrate ratio of 50:50 (DM basis) and were offered as a TMR for 4 weeks. Milk samples were taken at the end of the baseline and the end of the second, third, and last weeks of the test period for analysis of FA composition. Data were analyzed using a mixed model of SAS testing the fixed effects of diet, feeding phase, and their interaction. The model also included random effects of cow, block, and parity and with repeated measures within the cow using a spatial power covariance structure. **Results:** At baseline, both the dietary FA intake and profile of milk FA of all groups were similar. In the test period, increasing BBP content in the diet increased the intake of FA ($P < 0.001$). On average, CON cows consumed per day 77, 9, 96, 156 and 469 g of 16:0, 18:0, 18:1 n-9, 18:2 n-6 and total FA, respectively. 15BBP cows consumed 98, 17, 160, 185, and 621 g/d and 30BBP cows 129, 25, 227, 206, and 770 g/d, respectively. The average 18:3 n-3 intake ranged from 46-53 g/d. The percentages of total MUFA especially of the 18:1 FA (both cis and trans isomers) linearly increased with increasing dietary BBP level. Interestingly, the percentage of de novo FA (the sum of 4:0 – 14:0) remained similar among diets (32-34% of total FA) while the percentage of 16:0 linearly decreased with increasing BBP levels despite more 16:0 intake with the BBP diets. The milk 16:0 percentage in the BBP groups drastically dropped from 32.5-33.5% to 28-30% following the switch from baseline to the BBP diets (P phase < 0.001). Notably, only 30BBP elevated the percentage of conjugated linoleic acids (CLA) (0.53%) compared with CON (0.35%) ($P < 0.05$). The percentages of PUFA like 18:2 n-6 and 18:3 n-3 and the n-6:n-3 ratio remained unaffected by BBP and feeding phase. **Conclusions:** Feeding of BBP led to shifts in the milk FA profile to more 18:1 FA at the expense of 16:0. Inclusion of BBP at 30% of diet DM also increased CLA contents in milk fat, indicating its effect on ruminal biohydrogenation. Furthermore, the data suggest that with sufficient provision of energy and dietary FA there is a tight regulation of the proportions of de novo FA and PUFA in the milk FA profile in dairy cows.The authors thank S. Sharma (Vetmeduni Vienna) for the contribution to sample analysis.

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14.

In vitro digestibility and fermentation characteristics of fibres and fibre-rich feed components for pigs

In vitro Verdaulichkeit und Fermentationscharakteristika von Fasern und faserreichen Futterkomponenten für Schweine

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In pigs, regular supply with fibres may help to prevent dysbiosis and dysfunction of digestive processes. In this study, fermentation characteristics and in vitro dry matter (DM) digestibility (IVDMD) of fibre preparations and fibre-rich feeds were determined.

Methods: Lignocellulose with bark (LC+) and without (LC-), pulverized cellulose (PC), Aspergillus niger mycelium (ANM), lucerne chaff (LU), soybean hulls (SH), wheat bran (WB), and sugar beet pulp (SBP) were investigated. Pre-digestion of the substrates by body-own enzymes was carried out according to step 1 and 2 of the method proposed by Boisen and Fernández (1). For each of 7 runs of the subsequent batch-culture fermentation, faeces were taken from minimal 3 weaned piglets. Faeces plus physiological saline (1:5) were filtered and mixed with buffer 1:2 under CO_2 flush. The hygienized substrates were incubated in the ANKOM RF Gas Production System (0.4 g/30 mL; 7 replicates), both pre-digested and not pre-digested. The gas volumes were documented; concentrations of CH_4 and short chain fatty acids (SCFA) were measured by GC-FID. IVDMD was determined according to Noblet and Jaguelin-Peyraud (2). The substrates' physicochemical properties were analysed as described by Kyriazakis and Emmans (3). Statistical analysis was performed using fixed effects of substrate, pre-digestion, and their interaction, and a random effect of the run at a significance level of $P < 0.05$.

Results: Concentrations of DM (g/kg), crude protein (g/kg DM), and neutral detergent fibre (aNDFom; g/kg DM) were 933, 11, and 897 (LC+), 938, 5, and 918 (LC-), 939, 7, and 999 (PC), 904, 144, and 857 (ANM), 936, 151, and 474 (LU), 912, 112, and 707 (SH), 919, 163, and 508 (WB), and 921, 105, and 500 (SBP). Water binding capacity (g/g DM), water holding capacity (g/g DM), and the swelling capacity (%) were 6.7, 5.4, and 80 (LC+), 8.2, 6.2, and 44 (LC-), 14.3, 9.7, and 9 (PC), 4.4, 4.1, and 367 (ANM), 7.7, 8.0, and 110 (LU), 7.5, 6.8, and 240 (SH), 6.4, 6.4, and 59 (WB), and 10.4, 10.6, and 411 (SBP). Gas production (48 h) was 109, 92, 88, 72, 58, 56, 16, and 7 mL/g DM for SBP, WB, SH, LU, ANM, PC, LC+, and LC-, respectively. It was lower when the substrates had been pre-digested ($P < 0.01$: WB, SH, LU; $P > 0.05$: others). CH_4 increased throughout incubation. The greatest concentrations were achieved in PC, LU, and SH (2,251–2,236 µmol/L). The LCs were barely fermented; only 324–743 µmol CH_4/L were produced. Pre-digestion did not affect CH_4 production, because solutes sparsely contribute. Initial pH was 6.11 ± 0.0622. It decreased up to 5.12 (SBP), but remained unaltered with LCs. The C_2:C_3+C_4 fatty acid ratio was between 1.3 (WB) and 3.1 (PC) excluding the LCs. Only acetic acid was marginally produced from fermentation of LCs (0.85–1.45 mmol/L). Significant production of SCFA was found in SBP, SH, LU, WB, ANM, and PC. SCFA concentrations differed among the substrates ($P < 0.05$). Enzymatic pre-digestion mostly reduced the production of SCFA ($P > 0.05$). IVDMD (pre-digestion) was lower than 0.06 in ANM, SH, PC, and LCs, but 0.19, 0.22, and 0.37 in LU, SBP, and WB, respectively. IVDMD (fermentation; not pre-digested) ranged between 0.10 (PC) and 0.44 (WB). IVDMD (pre-digestion plus fermentation) was lowest in LC+ (0.07) and LC- (0.09) and greatest in SBP (0.55).

Conclusions: Among the tested fibre preparations, ANM had the greatest fermentation capacity and total IVDMD (0.28). The LCs remained widely unfermented. They might have positive effects on satiety and gut health when applied as bulk material.

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15.

Impact of cutting height on fermentation parameters and aerobic stability of corn silage

Einfluss der Schnitthöhe auf Fermentationsparameter und die aerobe Stabilität von Maissilage

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Increasing the cutting height of corn reduces the biomass yield but decreases the fiber content and has typically positive impact on fiber degradability and the energy content of corn silage (1). Consequently, there is continued research and practical interest in defining the optimal cutting height of corn harvested for silage. While the impact of cutting height on the nutrient content and animal performance is fairly well described, information concerning the impact of cutting height on fermentation parameters and particularly aerobic stability of corn silage are scarce. Therefore, this study was designed to compare the effect of practically applied cutting heights on fermentation parameters and the aerobic stability of corn silage.

Methods: Whole crop corn was harvested in early dough stage by hand from three different locations within a uniform plot. Chopping heights were 20, 30, 40, and 50 cm above ground. The corn plants were transported to the lab and chopped using a laboratory-scale chopper. Quadruplicate samples of each treatment were packed in cylindrical PVC mini silos (3.4 L volume) using a pneumatic press. The mini silos were sealed and stored at ambient temperature for 56d. After opening, silages were subsampled and analyzed for chemical composition, pH and concentration of fermentation acids. The remaining silage was placed in separate insulated containers and stored at room temperature for 13d. Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY, USA) were placed in the mid-layer of the silage in each container and in the room where the silos were stored, to record the heat loss from the silages as indicator for its aerobic stability. For data analysis, continuous temperature recordings were summarized for each day of the aerobic stability measurements. Data were analyzed using a mixed model procedure in SAS (Version 9.4). For the analysis of chemical composition data, pH, and the concentration of fermentation acids cutting height (20, 30, 40 and 50 cm) was included as fixed and mini silo as random effect. For the aerobic stability data, day and the interaction of cutting height and day was included as fixed effect. The effect of cutting height on the tested parameters was examined through linear and quadratic orthogonal contrasts. Significance was declared at $P<0.05$.

Results: As expected, the starch and energy content of the silage increased linearly in response to increased cutting height ($P < 0.05$). All samples were below pH 3.9, while cutting height had no effect on silage pH. Heat losses peaked on d 5 but aerobic stability was similar between treatments. Concentration of lactic acid was 3.77% in silage cut at 20 and 30 cm and decreased linearly ($P < 0.05$) to 3.57% (40 cm) and 3.38% (50 cm; all DM basis). Ethanol concentrations were the highest in silage cut 20 cm above ground (1.11% DM) and decreased linearly to 0.71% DM in silage cut 50 cm above ground ($P < 0.05$). Butyric acid was not detected in any of the silages. Surprisingly, the crude ash ($r = 0.77$) and acid detergent fiber content ($r = 0.67$) of the un-ensiled corn had the strongest positive correlation with the lactic acid concentration in the silages ($P < 0.01$).

Conclusions: Under the conditions of this experiment, aerobic stability of corn silage was not affected by differences in cutting height. Even though mean concentrations of ethanol were relatively low, ethanol increased in response to lower cutting height, which indicates that silage quality could be affected negatively by the activity of yeast when corn stands are cut low. It needs to be acknowledged that the corn was harvested at an optimal growing stage; however, the composition and activity of the epiphytic microflora can be impacted by the maturity of the plant as well as environmental factors. Therefore, future research should be directed towards the impact of cutting height on the quality of corn silage that is harvested later in the growing season under less favorable conditions.

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16.

Phytate degradation in pigs fed wheat-based diets and using different wheat genotypes

Phytatabbau bei Schweinen und bei Einsatz von verschiedenen Weizengenotypen im Futter

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The addition of microbial phytase to diets that did not contain plant phytase significantly increased the precaecal phytate (InsP6) degradation in pigs to 92% (1). The effect of plant phytase activity on gastrointestinal InsP6 degradation has barely been studied but total tract P digestibility is higher in wheat-based diets than in diets based on maize (2). Variation in plant phytase activity among wheat sources may affect InsP6 degradation in pigs, but this has not been studied to date. The objective of the present study was to measure precaecal and faecal InsP6 degradation in pigs provided with wheat-based diets. Two wheat crosses (W1: 3,700 FTU/kg and W2: 3,400 FTU/kg) containing genes of a high-phytase wheat mutant (3) were provided by W. von Borries-Eckendorf GmbH and compared with standard wheat (W3: 2,760 FTU/kg). Additional effects of added microbial phytase were also studied. **Methods:** The four experimental diets contained 40% wheat, 21.8% maize, 21% soybean meal, 12% rapeseed meal, 0.5% TiO_2 as the indigestible marker, and 4.7% oil and premixes. A mineral P source was not included. Total P and Ca concentrations of the diets were on average 5.6 and 6.5 g/kg dry matter. Three diets only differed in the included wheat source (W1-W3). Diet 4 contained W3 and was supplemented with 500 FTU/kg of a 6-phytase (W3+). Eight barrows with an initial body weight of 27 kg were fitted with a simple T-cannula at the distal ileum and assigned to the four dietary treatments in a completely randomized Double Latin Square design. Feed was provided twice a day in mash form and daily feed allowance was 4% of average body weight. The experiment included four periods of 12 days each. The first five days of each period were considered as diet adaption, followed by four days of semi-quantitative faeces collection. Ileal digesta was collected on the 10th and 11th day for 12 hours each. Concentrations of Ca and P were analysed using ICP-OES following wet digestion, inositol phosphates by HPIC following alkaline extraction, and myo-inositol by GC-MS. Data were analysed in a one-factorial analysis of variance using the Mixed Model of JMP Pro. **Results:** The analysed phytase activity in the four diets were (FTU/kg): 1,410 for W1, 1,370 for W2, 1,200 for W3, and 1,850 for W3+. Precaecal InsP6 disappearance was 48% in diets W1, W2, and W3, without significant differences among wheat varieties, and significantly higher in diet W3+ (79%). Correspondingly, precaecal P digestibility was higher in W3+ (53%) than in the average of W1-W3 (37%). Total tract P digestibility was almost the same as precaecal P digestibility in all diets although total tract InsP6 disappearance was 97% or higher in all diets. In the ileal digesta, the concentrations of InsP5, InsP4, InsP3, InsP2, and myo-inositol were not significantly different between diets containing W1, W2, and W3, but were significantly lower (InsP5) or higher (InsP2, InsP3, InsP4, myo-inositol) in W3+ than in the other treatments. **Conclusions:** Phytase from wheat caused a noticeable release of P from InsP6 in the gastrointestinal tract of pigs. Differences in phytase activity of wheat may not affect InsP6 degradation in pigs at an inclusion rate of 40% wheat in the diet. Nevertheless, InsP6 degradation and P digestibility can be further increased by addition of microbial phytase to wheat-based diets.

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17.

On the mechanism of performance enhancing effects of Rare Earth Elements in piglets.

Zum Mechanismus der leistungsfördernden Wirkung Seltener Erden beim Ferkel

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Rare Earth Elements (REE) have gained attention as potential growth promoters since a long time, first in China and later in Europe under Western feeding conditions (Rambeck and Wehr, 2005). Flachowsky et al. (2019) gave an overview on feeding studies and on possible implications of REE in animals. Lanthanum and cerium were shown not to accumulate in organs, muscle or bone following highly elevated oral supplementation of lanthanide citrate in weaned piglets (von Rosenberg et al., 2013) In 2019, EFSA declared a Lanthanum-Cerium-citrate mixture (Lancer) given to pigs as safe for the consumer and the environment. As a result, the European Commission very recently authorized this lanthanide citrate as a feed additive for weaned piglets in the category „zootechnical additives". (Official Journal of the European Union, October 2nd 2020 L319/5) In Switzerland, this feed additive was already authorized in 2003, and since then, it is on the feed market.

The mechanism behind this growth promoting and feed conversion improving effect is still unclear, but it was assumed that REE might exert their action locally on gut microbial populations (Tariq et al., 2020). Xiong et al. (2019) and others found more beneficial bacteria (Christensenellaceae and Ruminococcaceae) in the REE-supplemented group while some opportunistic pathogens (Proteobacteria and Campylobacter) were relatively suppressed. Fecal microbiota showed correlation with antioxidase, inflammatory factors, and average daily weight gain.

Furthermore, it was revealed recently that - in addition to the well-known Ca - methanol dehydrogenase - lanthanide - dependent enzymes exist in nature. The essential nature of REE for certain bacteria has been studied by the working group of Daumann (Daumann, 2019). Lanthanides are here neccesary for activating a bacterial redox cofactor (Pyrroloquinoline Quinone) that is important for the energy metabolism of these bacteria.

That might explain why lanthanides are biologically relevant and shed light on the decades-long assumption that REE might have an influence on the microbiome in the intestine and that the positive effects on the immune system and stable growth in piglets could be triggered by them. These findings make Rare Earth Elements promising and safe feed additives.

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18.

A meta-analysis on estimates of efficiency of calcium utilisation in ruminants

Eine Meta-Analyse zu Schätzwerten für die Calciumverwertbarkeit bei Wiederkäuern

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Calcium (Ca) is the most abundant mineral in the body of animals. Maintenance of Ca balance of lactating cows has become more challenging, owing to the increased milk performance and thus Ca requirement. The Ca requirement is determined by the net requirement, primarily the Ca secreted with milk, and estimates of the efficiency of Ca utilisation. Although the efficiency of Ca utilisation is commonly defined as the maximum proportion of Ca from the feed that the animal can use for covering the net requirement, estimates currently used by different committees are inconsistent. While the GfE [1] applies a value of 50%, other committees such as the NRC, the INRA or the ARC apply values between 30% and 68%. This wide range may be related to the fact that those suggestions disregard the Ca supply of the animal in relation to the Ca requirement. The objective of the present study was, based on published data, to obtain an updated, reliable estimate of the efficiency of Ca utilisation and excretion in relation to the Ca supply of the animal. It was hypothesised that Ca digestibility could be used as an estimate for the efficiency of Ca utilisation provided that Ca is supplied below the Ca requirement of the animal. **Methods:** The data set included 214 observations compiled from 34 studies on cattle and small ruminants obtained from literature (at maintenance or lactating, growing, and pregnant animals). Inclusion criteria were that at least data on Ca intake (CaIN), Ca faecal excretion (CaFE) and dry matter intake (DMI) had to be reported. Data on CaIN and CaFE were used to calculate standardised Ca digestibility corrected for faecal endogenous losses, i.e. 1 g Ca per kg DMI [1]. For lactating ruminants, a data subset was created, including data on CaFE, Ca in urine, and standardised Ca digestibility In order to relate the data to the Ca supply status of the animals, the net requirement was estimated as the sum of Ca secretion in milk and inevitable Ca losses [1] for lactating animals (cattle and small ruminants). As an approximation, the gross requirement was calculated assuming an efficiency of Ca utilisation of 50% [1] or 68% [2]. For the purpose of this analysis, the Ca supply was considered to be below the requirement when the difference between actual Ca intake and estimated Ca gross requirement was negative. Linear regression equations between the different Ca-related traits were determined. **Results:** The median of standardised Ca digestibility was 41% and it varied considerably between 9% and 88%. CaFE increased linearly with increasing Ca supply (slope = 0.996, $R^2 = 0.91$). Urinary Ca excretion was overall very low (mostly below 3 g/day), even when Ca supply was very high. Out of the 86 observations made for lactating ruminants, only 24 or 4 could be considered to be below the Ca gross requirement, depending on the assumed efficiency of Ca utilisation. The median values of standardised Ca digestibility for those data were 54% and 58 %, respectively. **Conclusions:** Each gram of CaIN exceeding the Ca requirement caused the Ca excretion in faeces to increase by one gram. Excretion of Ca in urine has only minor quantitative relevance and is independent of the level of Ca intake. Thus, standardised Ca digestibility can be used as a measure for the efficiency of Ca utilization of ruminants, provided that the Ca supply of the animal is below the requirement. Nevertheless, only a very limited number of studies have reported data for such conditions, thus, differentiation between Ca sources is not yet possible. Considering the low number of observations and variation associated with the presented estimates, they may be considered a confirmation of the value of the efficiency of Ca utilisation used by GfE [1], which is 50%.

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19.

Estimation of dietary phosphorus requirements of dairy cows during the dry period by means of a biomarker for bone resorption

Einschätzung des Phosphorbedarfs trockenstehender Milchkühe anhand eines Biomarkers für Knochenmobilisation

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Assessing phosphorus (P) requirements of cattle is challenging due to the incomplete understanding of the regulation of the P-homeostasis. Recent studies showed that bone mobilization is an important counter-regulatory mechanism in P-depleted ruminants. In this study we attempted estimating P-requirements of dry dairy cows by assessing bone mobilization activity with varying dietary P supply.

Methods: Twenty late pregnant dairy cows were assigned to either a diet with adequate (AP;3.5 g/kg DM) or low P (LP;1.5 g/kg DM) content. All cows received the AP-diet during a 2-weeks acclimation, before switching cows to their corresponding diet. Blood was obtained after acclimation (T_0), and 14 (T_1) and 24 days (T_2) later to determine the CrossLaps concentration ([Ctx]) as marker for bone resorption. The change of [Ctx] from T0 (ΔCtx) was calculated for T_1 and T_2, and the daily P- and Ca-intake per cow (Pcow) and per kg BW (PkgBW) was calculated. Correlation- and regression analyses were conducted to identify the range of dietary P supply that was associated with enhanced bone mobilization.

Results: ΔCtx, but not Ctx was significantly associated with Pcow (T_1:r=-0.63, P=0.005; T_2:r=-0.80, P=0.0003) and PkgBW (T_1:r=-0.69, P=0.001; T_2:r=-0.79, P=0.001). Regression analysis including dietary P and Ca supply yielded associations of ΔCtx only with Pcow and PkgBW. Scatter plot examination revealed a marked increase of ΔCtx with PkgBW<30 mg/kg BW and Pcow<25g/cow.

Conclusions: These results indicate that dietary P supply above 30 mg/kg BW does not trigger counter regulation in the form of bone mobilization in dry dairy cows.

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20.

Are porcine bristles an indicator for dietary copper excess?

Sind Schweineborsten ein Indikator für einen ernährungsbedingten Kupferüberschuss?

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Since the EU-wide ban of in-feed antibiotics for weaned piglets, different alternatives are used such as elevated dietary levels of copper due its antimicrobial potential. The downside are elevated levels in manure and thus the environment as well as a potential increase in antibiotic resistance of certain gut microorganisms. In order to monitor such enhanced levels, the analysis is usually carried out in feed, blood or faeces. However, as copper can also be enriched in hair, the present study aimed to investigate whether bristles are a potential sample matrix for monitoring copper supply.

Methods: In two subsequent feeding trials, 2 x 80 weaned barrows (6.39 ± 1.1 kg, 7.81 ± 1.2 kg live weight), were group-housed (4 pigs/pen) and equally assigned to one of four diets with graded copper levels (5 pens/diet) during 5 weeks of rearing: 6 mg/kg feed (Cu6), 25 mg/kg feed (Cu25), 150 mg/kg feed (Cu150) and 250 mg/kg feed (Cu250). In order to achieve the different copper levels, a standard rearing diet was enriched with appropriate amounts of copper sulphate. After rearing, pigs were relocated to a fattening unit and group-housed according to their diet group during rearing (n=20 pigs/pen, 4 pens). All fatteners received the same diet with the recommended copper level. During the experimental period, bristles were sampled at regular intervals: approximately 0.5 g were closely clipped to the skin on days 1, 15, 28 and 56 after start of the trial. Therefore, the first three samplings were during rearing with copper supplementation, whereas the last sampling was during fattening without graded copper levels. Concentrations of copper (Cu) in diets and bristles were analysed on an ICP-OES according to VDLUFA (2006). No analysis results are available for day 1 of trial1 because the amount of bristles was not sufficient for analysis. The results were analysed using Kruskal-Wallis-Test in SAS 9.4.

Results: The analysed copper concentration of the four rearing diets (Cu6, Cu25, Cu150, Cu250) in trial1 were 6, 32, 174 and 280 mg Cu/kg feed, and 18, 29, 158, 281 mg Cu/kg feed in trial2. The copper concentration in the fattening feed was 21 mg Cu/kg feed in trial1 and 15 mg Cu/kg feed in trial2. In both, trial1 and trial2, the median copper concentration in bristles significantly increased from day 15 to day 28 and day 1 to day 28, respectively, in groups Cu150 (trial1: 27 | 31 mg Cu/kg DM; trial2: 18 | 27 | 56 mg Cu/kg DM) and Cu250 (trial1: 30 | 35 mg Cu/kg DM; trial2: 19 | 36 | 70 mg Cu/kg DM). Only slight variations in concentrations are seen in groups Cu6 (trial1: 16 | 16 mg Cu/kg DM; trial2: 18 | 15 | 19 mg Cu/kg DM) and Cu25 (trial1: 18 | 17 mg Cu/kg DM; trial2: 17 | 17 | 25 mg Cu/kg DM). In bristle samples from fattening pigs, there was a significant reduction in the median Cu concentration from day 28 to day 56 in groups Cu150 (trial1: 31 | 19 mg Cu/kg DM; trial2: 56 | 21 mg Cu/kg DM) and Cu250 (trial1: 35 | 20 mg Cu/kg DM; trial2: 70 | 24 mg Cu/kg DM). The Cu concentration in the samples from groups Cu6 (trial1: 16 | 19 mg Cu/kg DM; trial2: 19 | 21 mg Cu/kg DM) and Cu25 (trial1: 17 | 21 mg Cu/kg DM; trial2: 25 | 20 mg Cu/kg DM) did not significantly vary.

Conclusions: The study has shown that the analysis of Cu concentration in pig bristles is possible and that graded levels of inorganic copper supplemented via feed are detectable in the bristles. However, the effect of supplemented Cu is also reversible, as it was shown that after four weeks without supplementation, the concentration in bristles of pigs from all four groups was similar. In future studies dealing with the supply of pigs with Cu, pig bristles should be used as an easily obtained, well storable analysis matrix with a high validity in the results.

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21.

Economic comparison between dicopper oxide and organic copper sources

Ökonomischer Vergleich zwischen Kupfer(I)-oxid und organischen Kupferverbindungen

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Copper (Cu) is commonly used at growth-promoting level in piglets feed but environmental concerns and the increasing occurrence of bacterial resistance to antibiotics lead to a significant reduction of the maximum total authorised level of Cu in Europe. Before 2019, the maximum Cu concentration in complete feed for piglets was 170 ppm until 12 weeks of age. Nowadays, Cu level in piglet diets cannot exceed 150 ppm until 4 weeks post-weaning and 100 ppm until 8 weeks post-weaning. In this new regulatory context, efficiency of potentially better available Cu sources has been evaluated.
Methods: Growth performance of 660 piglets, weaned at 26 days, was measured during the starter period (40-72 days) in a commercial farm located in Lovászpatona (Hungary). The animals were allocated into 30 pens (22 piglets per pen) divided in 3 treatment groups: 90 ppm of Cu from MHA-chelated Cu, Cu-glycinate or dicopper oxide (CoRouge®) sources. Piglets were weighted at the beginning and at the end of the trial. Feed intake was measured and FCR was calculated. One-way Analysis of Variance (ANOVA) was used to evaluate the source-effect, using the pen as experimental unit. Economic gain was evaluated, according to feed cost, global feed intake and global weight gain per pen.
Results: There was no significant source-related effect for growth performance ($p > 0.1$), but weight gain was numerically better in pigs fed with dicopper oxide. Concerning feed supplementation costs, investment for dicopper oxide was at the same level compared to Cu-glycinate and below the MHA-chelated Cu. Feed efficiency of piglets fed dicopper oxide was numerically better compared to the Cu-glycinate group and similar to the MHA-chelated Cu group, leading to improved economical results (+1.35% and +1.1% compared to MHA and to glycinate, respectively).
Conclusion: When supplemented under a typical Hungarian context, dicopper oxide can bring economic benefits compared to chelated Cu sources.

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22.

Dicopper oxide might promote a positive shift on intestinal microbiota in weanling pigs

Kupfer(I)-oxid kann bei Absatzferkeln eine positive Verschiebung der Darmmikrobiota fördern

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Copper (Cu) shows a growth-promoting effect on weaned piglets and growing pigs. Supplemented at supranutritional levels, it can increase feed intake and weight gain. Its mechanisms of action are not fully elucidated but the pre-absorption modulation of the intestinal microbiota is one of the main hypotheses, related to its strong antimicrobial properties. **Methods:** The study was performed to evaluate the effects of 2 Cu sources ($CuSO_4$ and Cu_2O) on the intestinal microbiota of weanling pigs. A total of 180 piglets (Topigs x Pietrain), weaned at 28 days and having an initail live weigth of 9.18 ± 0.11 kg, were allocated into 2 treatments: 150 ppm of Cu from $CuSO_4$ or Cu_2O (CoRouge®), with 8 replicates per treatment and 8 piglets per pen. At the end of the trial (35 days after weaning), 1 pig per pen was slaughtered and the proximal and distal content of the ileum was collected for microbiota evaluation (PCR, 16S rRNA gene method). Statistical analysis was performed with RStudio v.3.5.1. **Results:** An increase of 8.6% in body weight was observed when pigs were fed 150 ppm Cu_2O compared with pigs fed $CuSO_4$. Bacterial diversity and species richness, determined by the Shannon index, were greater ($P < 0.05$) in pigs fed 150 ppm Cu_2O than pigs fed $CuSO_4$ in the proximal, but not in the distal portion. Whatever the source, alpha diversity decreased from the proximal to the distal portion. Pigs fed 150 ppm $CuSO_4$ had greater abundance (FDR ≤ 0.2) of Enterobacteriaceae and Clostridium sense strictu populations, and lower abundance of Bifidobacteriaceae than pigs fed Cu_2O in the proximal portion. No significant difference in coliforms count in the distal portion was observed. Furthermore, there was no difference in Lactobacillus abundance in relation to the source. **Conclusion:** In comparison to $CuSO_4$, higher diversity and lower abundance of coliforms in the proximal intestinal segment could partially explain the improvement in weight observed in pigs fed with Cu_2O.

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23.

Effects of dietary Ca and microbial phytase on zinc utilization in broiler chickens

Effekte von zugelegtem Ca und mikrobieller Phytase auf die Verwertung von Zink in Masthähnchen

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The bioavailability of zinc (Zn) is related to its solubility in the gut, which is determined by source's physicochemical properties and dietary factors, e.g., calcium (Ca) and phytase. The aim of this study was to evaluate the effects of microbial phytase (PHY), dietary Ca and Zn sources: oxide (ZnO; HiZox®) vs sulfate ($ZnSO_4$) on performance, bone mineralization and Zn concentration in tibia and plasma in broiler chickens. **Methods:** Eight diets were fed to 144 male broilers (Ross 308) from 8 to 21 d according to a 2×2×2 factorial arrangement: 2 levels of Ca (6 and 10 g/kg), 2 sources of Zn (ZnO and $ZnSO_4$) and 2 levels of PHY (0 and 750 FTU/kg). Animals were randomly assigned in the treatments and individually housed in cages. Diets contained 27 ppm native Zn and 2.3 g/kg of phytic phosphorus (PP). 23 ppm of Zn from ZnO or $ZnSO_4$ were supplemented and two non-phytic phosphorus (nPP) levels of 4.5 or 3.2 g/kg were defined considering inclusion of 0 or 750 FTU/kg, respectively. At the end of the trial, a blood sample was taken from the occipital sinus of each bird for determining Zn concentration in plasma by ICP-OES. Immediately after, euthanasia was conducted and the right tibia was dissected from each bird to asses bone characteristics (weight, ash, strength) and Zn content (ICP-OES). Data was subjected to a 2×2×2 ANOVA analysis. **Results:** Analysed dietary Ca was slightly lower than expected in the low-Ca diets (5.3 vs 6.0 g/kg), on target in the high-Ca ones. Body weight (BW) gain and feed efficiency were positively affected by ZnO compared to $ZnSO_4$ (+27g, $P<0.01$, +0.03 points, $P<0.001$, respectively). High-Ca diets improved feed intake (+32g, $P<0.01$) and final BW (+25g, $P<0.05$). A lower gain was observed when PHY was supplemented to low-Ca diets probably due to a Ca deficiency (Ca* PHY, $P<0.05$). Tibia weight, ash and length were positively affected by ZnO compared with $ZnSO_4$ ($P<0.05$, $P=0.062$ and $P<0.01$) and is linked to the significant effect of ZnO on growth. High Ca affected positively tibia weight, ash weight (g) and content (%) and breaking strength ($P<0.0001$) illustrating the requirement in Ca for bone growth and mineralisation. The skeleton being a main site of storage of Zn, high-Ca improved tibia Zn content and deposition whatever the Zn source (+4.9 mg/kgDM, $P<0.01$, +38µg, $P<0.0001$). PHY supplementation increased tibia Zn deposition (+53µg, $P<0.0001$) as well as tibia Zn content dependently of the Zn source (PHY *Zn source, $P<0.05$) with an improvement of 21 and 15 mg/kgDM in birds fed $ZnSO_4$ and ZnO, respectively. Plasma Zn, a short-term indicator of Zn availability, also increased with PHY supplementation ($P<0.001$). There was a trend for the interaction PHY*Zn source ($P=0.067$) as PHY increased plasma Zn only in birds fed ZnO. **Conclusion:** The effect of PHY on Zn availability seems to depend on the Zn source but also on the response criterion. ZnO allowed better performance response of broilers. Compared to sulfate, dynamics of Zn absorption and deposition may be linked to Zn solubility and interaction with phytate; features that can explain the differential response observed between Zn in plasma and in bone; hence deposition seems more gradual. Dietary Ca level affects Zn utilization independently of the Zn source whereas the effect of PHY depends on it.

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24.

The effect of maternal dietary protein malnutrition during pregnancy on the circulatory metabolome in exposed neonatal piglets

Effekt einer imbalanten mütterlichen Proteinzufuhr während der Trächtigkeit auf das Kreislauf-Metabolom exponierter neugeborener Ferkel

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Protein malnutrition during pregnancy has been associated with lower offspring birth weight and impaired growth and development (1). To date combined data on growth and circulatory blood parameters and targeted metabolite profiles during the first 24 hours of life in piglets are scarce. The objective of this study was to characterize the circulatory clinical blood parameters and targeted metabolite profile of newborn piglets from mothers fed a low (n = 14, LP-HC; 6.5% protein, 68% carbohydrates), high (n = 16, HP-LC; 30% protein, 39% carbohydrates) or isoenergetic adequate protein to carbohydrate diet (n = 14, AP; 12.1% protein, 60% carbohydrates) during pregnancy. We hypothesize that prenatal exposure to protein malnutrition is associated with alterations in plasma hormone and metabolite profiles. **Methods:** German landrace piglets (AP, n = 53; LP-HC, n= 49; HP-LC, n = 58) born to gilts fed an AP, LP-HC or HP-LC diet throughout pregnancy were selected. Offspring were nursed by their respective dams, weighed, and at 1 d of age, serum and plasma was collected for analyzing hormone and metabolite concentrations. Clinical blood parameters were measured in serum (high- and low-density lipoprotein cholesterol concentrations), whilst insulin, glucagon, glucose, non–esterified fatty acids, triglycerides, total cholesterol and urea were determined in plasma. Targeted metabolite analysis was conducted using a subset of plasma samples (n = 8 / diet), using the AbsoluteIDQTM p180 Kit (Biocrates), enabling 188 metabolites to be quantified (2). Targeted metabolite data underwent generalized logarithm transformation (glog2), normalization and assessment using MetaboAnalyst (v 4.0). All data was then analyzed using the MIXED procedure of SAS. Clinical blood parameter least square means were separated using the Tukey test ($P < 0.05$), whilst significance levels of the targeted metabolite least squared means were calculated using false-discovery-rates. **Results:** At birth, LP-HC and HP-LC offspring were lighter than AP and by 1 d only LP-HC were lighter. The clinical blood parameters showed LP-HC plasma concentrations of insulin ($P = 0.02$) and urea ($P = 0.06$) were less than, and the glucose:insulin ratio ($P = 0.03$) greater than in HP-LC offspring. No differences were observed in the targeted metabolite profiles were observed. **Conclusion:** The changes observed were modest in comparison to diet-related changes reported from companion studies of fetal and maternal development in this model, indicating substantial pre-natal adaptation by the sow (and placenta) to protect the developing fetus.

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25.

Effect of DL-methionine supplementation on tissue and plasma antioxidant status and concentrations of oxidation products of cholesterol and phytosterols in heat-processed thigh muscle of broilers

Auswirkungen einer DL-Methionin Supplementierung auf den antioxidativen Status von Plasma und Geweben sowie die Konzentrationen der Oxidationsprodukte von Cholesterol und Phytosterolen in erhitztem Oberschenkelmuskel von Broilern

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The occurrence of oxidative stress is a general problem in high yielding farm animals. Oxidative stress not only affects animal health, but free radicals generated under oxidative stress conditions can also promote the formation of lipid oxidation products¸ which are detrimental for the consumer. Therefore, prevention of oxidative stress by improving the antioxidant system has high priority in farm animal management. Methionine (Met) is a precursor of glutathione (GSH), one of the major antioxidants in the body. In the present study, we investigated the hypothesis that feeding diets with an excess of Met in relation to the requirement for optimum growth improves the antioxidant status by enhancing the formation of GSH in broilers.

Methods: Male Cobb-500 broilers (n=72) were allotted to three groups and phase fed three basal diets consisting mainly of corn and soybean meal during days 1-10 (phase I), 11-21 (phase II) and 22-35 (phase III). The concentrations of Met + cysteine (Cys) in the basal diets agreed with the recommendations of National Research Council (NRC, 1994). However, standardized ileal digestible (SID) Met + Cys levels were about 15-20% below the recommendations of the breeder and 10% lower than those recommended by AMINOChick 2.0 (Evonik Nutrition & Care GmbH, Germany). The control group received the basal diets, the two treatment groups (DLM 1, DLM 2) received the basal diets supplemented with DL-methionine (DLM) in two different concentrations in order to exceed recommendations of NRC for Met + Cys by 15-20 and 30-40%, respectively. The SID Met + Cys concentrations in the DLM 1 diets were close to the recommendations of the breeder, and AMINOChick 2.0. As parameters indicative of the antioxidant system, the concentrations of vitamin C, tocopherols, GSH and thiobarbituric acid-reactive substances (TBARS) in plasma or blood, liver and thigh muscle were determined. Moreover, the concentrations of oxidation products of cholesterol (COPs) and phytosterols (POPs) in heat-processed (170°C for 50 min in a drying oven) thigh muscle were determined by gas chromatography-mass spectrometry. The data of the three groups were analyzed by ANOVA.

Results: The three groups of broilers did not differ in body weight gains, feed intake and feed conversion ratio. There were only minor differences in the concentrations of total tocopherols, vitamin C and TBARS in plasma, liver and thigh muscle. However, broilers of groups DLM 1 and DLM 2 had higher concentrations of GSH in liver and thigh muscle than broilers of the control group ($P<0.05$). Moreover, broilers of groups DLM 1 and DLM 2 had lower concentrations of individual (7α-hydroxy cholesterol, 7β-hydroxy cholesterol, 7-keto cholesterol) and total COPs in heat-processed thigh muscle than broilers of the control group ($P<0.05$). Broilers of groups DLM 1 and DLM 2 had also lower concentrations of several POPs (7α-hydroxy campesterol, 7β-hydroxy campesterol, 7α-hydroxy sitosterol, 7β-hydroxy sitosterol) in heat-processed thigh muscle than broilers of the control group ($P<0.05$). There were significant inverse correlations between the concentration of GSH in thigh muscle and the concentrations of individual and total COPs in heat processed thigh muscle ($P<0.05$), suggesting that COP formation in broilers of groups DLM 1 and DLM 2 was reduced by increased GSH concentrations.

Conclusion: The study shows that supplementation of DLM in excess of the requirement for Met + Cys given by NRC, but in line with SID Met + Cys recommendations from the breeder and industry (AMINOChick, Evonik Industries), causes an increase of the concentration of GSH in the liver and thigh muscle, and reduces the formation of COPs in thigh muscle during heat processing. Minimum concentrations of COPs were already observed in group DLM 1, whereas a higher supply of DLM given in group DLM 2 did not further reduce the concentrations of COPs. As COPs have detrimental effects on human health, including the development of atherosclerosis and cancer, the reduction of COP concentration might be regarded favorably with respect to food safety.

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26.

Amino acid patterns of rumen bacteria and protozoa of cattle fed typical rations

Aminosäuremuster ruminaler Bakterien und Protozoen von Rindern bei typischen Rationen

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Because microbial protein accounts for a considerable supply of amino acids (AA) absorbed from the small intestine of ruminants, it seems indispensable to include microbial AA patterns in protein evaluation systems. Several studies have summarized literature data on this topic, with the latest being published in 2017 for lactating dairy cattle (1). As a general observation, protozoal protein is higher in Lys and lower in Ala concentration than bacterial protein (1, 2). Moreover, differences between liquid- (LAB) and particle-associated bacteria (PAB) have been reported for dairy and beef cattle fed rations with a concentrate inclusion of up to 85% (1, 2). However, it is controversial if the composition of the ration affects the AA composition of microbial crude protein. For this reason, a review of published literature was performed limiting included studies to reports on cattle and to mixed forage-concentrate rations with a maximum of 60% concentrate (dry matter basis) in order to obtain a result representative of rations common on dairy farms in Central Europe.

Methods: Three datasets (LAB, PAB, protozoa) were built from studies on ruminating cattle. According to Sok et al. (2017) Met and Cys were only included, when studies described adequate protection of sulfhydryl groups during sample hydrolysis. Within each microbial fraction, values differing from the mean with more than the twofold standard deviation were discarded (1). As Trp was only rarely analysed, mean Trp proportions were calculated for each of the microbial fractions and used as standard values in all following calculations. This procedure yielded a dataset for LAB, PAB, and protozoa, including 40, 24, and 18 observation means from 16, 9, and 8 studies, respectively. Descriptive statistics were performed on each dataset, and differences in AA pattern between LAB, PAB, and protozoa were calculated in SAS (version 9.4) using the GLM procedure. The microbial fraction was considered to represent the main effect und differences between fractions were determined using the Tukey-Kramer-test. Type 1-analysis was chosen to analyse the total sum of squares and account for the unequal sample size. Differences with $p < 0.05$ were considered significant.

Results: The reported proportions of an individual AA (g AA/100 g total AA) in individual microbial fractions varied considerably with minimum values accounting for only 50% of the calculated mean up to 172% of the mean as was the case for Cys in LAB. Greatest consistency was found for Leu and Ser in protozoa (87% to 105% and 95% to 112% of the means, respectively). Proportions of Cys, His, Leu, Met, Pro, Tyr, and Val were similar in LAB, PAB and protozoa. Alanine and Arg were higher in both LAB and PAB than in protozoa whereas protozoa had higher Asp, Glu, Ile, and Lys proportions. Differences between bacterial fractions were less numerous and consisted of lower Phe and higher Thr proportions in LAB than in PAB.

Conclusion: The proportions of numerous AA within individual microbial fractions vary within a large range. In contrast to Sok et al. (2017) there were only few and minor differences in the AA patterns of LAB and PAB when rations were limited to those that contained less than 60% concentrate. Calculated means for each fraction were in good agreement with values provided by recent literature.

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27.

Histomorphological development of the jejunum in low birthweight and normal birth weight piglets with or without glutamine supplementation during suckling period

Histomorphologische Entwicklung des Jejunums bei Ferkeln mit niedrigem Geburtsgewicht und normalem Geburtsgewicht mit oder ohne Glutaminsupplementation während der Säugeperiode

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Improved sow breeding techniques have led to increased numbers of low birth weight (LBW) piglets. The jejunum plays an important role in nutrient absorption and innate immune defense (1) and its development and function may be impaired in LBW piglets (2). Supplemental glutamine (GLN) has been shown to improve intestinal structure in weaning and post-weaning piglets (3); however, the influence of GLN during the early neonatal period has not been assessed. We hypothesize that GLN improves jejunal development and morphology in suckling LBW piglets. **Methods:** At birth, pairs of LBW (0.8 - 1.2 kg) and normal (NBW, 1.4 - 1.8 kg) male littermates born to gilts were selected. Approximately 24 hours after birth, litters were standardised to 12 piglets and experimental piglets were assigned to one of two isonitrogenous supplementation groups: GLN = 1 g/kg bodyweight; or alanine (ALA) = 1.22 g/kg. Thus, four different groups were studied: (LBW+GLN; NBW+GLN; LBW+ALA; NBW+ALA) (n = 12 / group). Piglets were orally supplemented 3 x daily (7:00, 12:00, 17:00) until d 12. Piglets were suckled by their respective dam for the duration of the study and had access to creep feed from d 14. Subgroups were euthanized at d 5, 12 and 26 (n = 48 / time point) and a section of mid-jejunal tissue was fixed in formalin. Histological examination of tissue morphology and neutral, acidic and mixed mucins was performed by Alcian blue pH 2.5 -periodic acid Schiff staining and images examined with the cellSens imaging software. Data were analyzed by SPSS using multivariate ANOVA F-test followed by Tukey post hoc test. **Results:** Age comparisons showed for all supplementation groups villus height (VH) increased from d 5 to 12 and decreased from d 12 to 26 ($P < 0.01$), crypt depth (CD) increased from d 5 to 12, and 12 to 26 ($P < 0.01$). The VH:CD ratio decreased from d 5 to 12, and d 12 to 26 for all groups except ALA+NBW which increased from d 5 to 12 ($P < 0.05$), and decreased from 12 to 26 ($P < 0.01$). Between d 5 and 12 the total amount of goblet cells in crypts as well as the number of NA (neutral and acidic mucins) in villi and crypts per 1mm basement membrane decreased ($P < 0.01$). A significant increase in the number of Acid (acidic mucins), Neu (neutal mucins), NA and a higher total number of goblet cells in villi could be observed from d 12 to 26 ($P < 0.01$). The same happend to the number of Neu, NA and the total number of goblet cells in the crypts ($P < 0.01$) only the number of Acid mucins decreased in crypts from d 12 to d 26 $P < 0.05$). The total number of goblet cells in villi and crypts decreased from d 5 to 12 ($P < 0.01$) and increased from d 12 to 26 ($P < 0.01$). ALA supplementation led to a higher number of Neu mucins at d 26, NA mucins at d 5 and d 26 and a higher number of total goblet cells at d 26 in the villi ($P < 0.05$). Neither Supplementation (GLN vs ALA) nor birth weight or their combination had further main effects on the investigated parameters. **Conclusion:** The results show that LBW may not be associated with impaired jejunal morphometric development compared to NBW littermates, and that GLN supplementation has no observable effect on the measured parameters. Age appears to have the most significant impact independent of supplementation and birth weight.

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28.

Effects of supplementation of diets for high-producing dairy cows in mid lactation with rumen protected methionine

Zum Einfluss einer Ergänzung von Rationen für hochleistende Milchkühe in der 2. Laktationshälfte mit pansengeschütztem Methionin auf Futteraufnahme und Leistung

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Methionine and lysine are considered to be the first limiting amino acids in dairy cows. Literature data on the effects of supplementation of dairy cow diets with rumen protected methionine on milk and milk protein yield is, however, inconsistent. Besides performance level or stage of lactation, reasons may be regional characteristics as breed of animals or fodder base and diet composition. In this context the present study was conducted to evaluate effects of rumen protected methionine on feed intake and performance of Fleckvieh and Brown Swiss cows fed grass and maize silage based diets.
Methods: The feeding trial involved a total of 48 dairy cows (36 Fleckvieh, 12 Brown Swiss) at day 102.±50 of third lactation. Cows were divided into two groups (control and methionine) according to actual feed intake, milk yield, and milk constituents. Cows were fed total mixed rations mainly based on maize silage, grass silage and straw/hay (36, 21, and 5 % of DM) which were balanced for concentrations of utilizable crude protein (uCP) at duodenum, ruminal nitrogen balance (RNB) and net energy for lactation (NEL). Diets for the methionine group were supplemented with rumen protected methionine (Mepron®, 0.06 % of DM). The experimental period lasted for 12 weeks. Individual feed intake and milk yield were recorded daily, milk constituents were measured weekly. BCS, RFD, and body weight were recorded at the start, the mid and the end of the experiment. Supply and requirement of metabolizable amino acids were calculated using the software AMINOCow®. Data were analysed by a one-factorial model using the GLM procedure of SAS.
Results: Mean dietary CP concentration of 15.2 and 15.4 % of DM was lower than planned (16.0 % of DM) in the diets of control and methionine group, what resulted in a slightly negative RNB of -0.8 g/kg DM and calculated negative amino acid balance for metabolizable methionine, lysine, histidine and leucine in diets for both groups. There was no difference in daily DM intake between the feeding groups (27.1 and 26.8 kg/day for control and methionine group). Daily energy and uCP intakes were similar for treatments, but intake of metabolizable methionine was higher (P<0. 01) in methionine (60 g/day) compared to control group (50 g/day). Milk yield for control and methionine group was 41.3 and 42.1 kg/day (P=0.64) and ECM yield was 40.8 and 41.7 (P=0.56), respectively. Milk protein concentration (3.69 and 3.67 %) and daily milk protein yield (1.52 and 1.54 kg/day) was similar for control and methionine groups (P=0.73 and 0.68). No effects on development of body condition traits were observed.
Conclusions: Dairy cows are able to maintain a very high production level when they are fed diets with a slightly negative RNB and metabolizable Met, Lys, His, and Leu balance but adequate in uCP, metabolizable protein and NEL concentration, presupposed a high DM intake is achieved. Under these conditions, rumen protected methionine at rates of 0.06% of DM did not increase performance of dairy cows in mid lactation significantly. In contrary, methionine improved cow's health and performance significantly in a number of transition phase trials. Such effects of methionine as a functional amino acid seem to be more pronounced at the onset of lactation than in mid lactation.

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29.

A combination of rumen-protected lysine and methionine increases blood serum concentration of certain essential amino acids in growing Fleckvieh bulls fed with a protein deficient diet

Die Kombination von pansengeschütztes Lysin und Methionin erhöht die Konzentration einzelner essentieller Aminosäuren im Blutserum bei proteinunterversorgten Fleckviehbullen

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A reduction in dietary crude protein (CP) and concurrent supplementation with rumen protected (RP) amino acids (AA) is the most promising method to increase protein efficiency and reduce nitrogen losses. The results of previous studies showed that RP lysine (Lys) could partly compensate for growth performance in Fleckvieh bulls fed with protein deficient diets (1), whereas RP methionine (Met) had no significant effects on zootechnical performance (2). The present study investigates the effect of a combination of RP Lys and Met on blood serum AA status of growing Fleckviehbulls fed with a protein deficient diet. **Methods:** This experiment comprised 67 German Fleckvieh bulls with an average age of 156 days and 223 kg live weight at d0 (LW0) of the experiment. The bulls were randomly allocated to three different diets. A diet sufficient in crude protein (14% CP of DM) served as control (CON; n=22). The second diet (NEG; n=22) was reduced in CP (11% CP of DM), while the third diet (LYS; n = 23) was equivalent to NEG but supplemented with rumen-protected Lys (0.42% Lys in DM). In order to keep the Met level constant and to avoid potential limitations by this presumably second limiting essential AA, RP Met was added at 0.11% of DM to NEG and LYS. All diets were formulated on an isoenergetic level (11.64 MJ ME/ kg DM). The animals were fed ad libitum up to d119. Starting with experimental d63, a balanced number of bulls were slaughtered from each group at weekly intervals until d119. Blood samples were collected from the jugular vein at experimental d27 and d62 and at each slaughtering date and immediately centrifuged for 10 min at 10,000G to separate the serum. The serum was then frozen at -20°C. AA in blood serum was analyzed using LC-ESI-MS/MS(MRM) measurement. Statistical analyses involved analysis of covariance (group, days until slaughter (DUS), LW0, group*DUS), multiple comparisons of means (SNK test) and orthogonal contrasts using SAS 9.4. **Results:** The sum of circulatory AA as well as the sub clusters of essential and non-essential AA were not significantly different between groups receiving either RP Met or a combination of RP Lys and Met but both groups were significantly lower in these parameters than the CP-adequate positive control group ($p<0.001$). Serum Lys concentration of protein sufficient group and RP Lys supplemented group were on the same level (CON: 259 µmol/l; LYS: 249 µmol/l) while Lys concentration of NEG was significantly reduced (NEG: 196 µmol/l; $p = 0.0125$). Although both CP reduced diets contained the same amount of Met, animals fed with RP Lys had also higher concentrations of serum Met ($p = 0.0062$). **Conclusion:** Supplementation of RP Lys together with Met increased the Lys and Met status of growing Fleckvieh bulls. This might partly explain the earlier observed gradual compensation of performance losses related to insufficient CP supply (1). This suggests that a proper combination of RP AA might be able to reduce the necessary total CP load of beef cattle diets. However, the optimum ratio of dietary CP and supplemented AA has yet to be evaluated.

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30.

Effects of temporary overdosage of branched-chain amino acids on weaned piglets of different weight categories

Effekte einer temporären BCAA-Überdosierung auf Absetzferkel unterschiedlicher Gewichtsklassen

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Besides its role as an essential protein component, leucine (Leu) has several other metabolic functions such as activation of protein synthesis. This property makes it an interesting amino acid (AA) for livestock production. However, Leu surplus stimulates the degradation of all three branched-chain amino acids (BCAA), causing peripheral imbalances among Leu, valine, and isoleucine and depriving feed intake and growth performance in consequence (1). The current experiment aimed to evaluate the effect of a combined surplus provision of all three BCAA in low protein diets on performance of early-weaned pigs divided into low-weight und high-weight groups. A crucial question was, if a combined surplus of all three BCAA might enhance performance by supporting protein synthesis or whether imbalances with other non-dispensable AA would result in an impairment of growth rates.

Methods: A total of 528 commercially reared, male and female piglets [(Landrace x Large White) x Pietrain] were weaned at the age of 21 days and blocked by body weight. Within the blocks, the piglets were randomly allocated into six different groups (n=88) without adaptation time. Piglets were fed ad libitum one of four mashed diets based on corn, barley and soybean meal, and supplemented with free AA to a constant Leu: Valine: Isoleucine ratio (100:70:53): 1) PC (15 % CP, positive control), 2) T1-150 & T2-150 (16 % CP, BCAA provided by 150 % of piglets' estimated requirement – T1 for one week, T2 for two weeks) , 3) T1-200 & T2-200 (16 % CP, BCAA provided by 200 % of piglets' estimated requirement – T1 for one week, T2 for two weeks), and 4) HC (17 % CP, all essential AA and arginine provided by 150 % of piglets' estimated requirement). Piglets allocated to T1 groups received the extra supplementation for one week. During the second supplementation week, T1 piglets received the PC feed. During the supplementation period, performance was documented weekly. Thereafter, all piglets received the same commercial Starter-diet for 21 days and were weighed again 35 days after weaning. Data were analyzed by General Linear Model ANOVA, using SPSS Statistical Software (IBM SPSS Statistics Standard 25, Armonk, NY, USA). In case of significant effects ($p<0.05$), means of the groups were compared by Hochbergs GT2-test.

Results: Significant effects on body weight and daily gain were detected at different times for the two weight categories. Low-weight piglets responded earlier to the dietary treatments, than high-weight piglets. Low-weight piglets continued to benefit from receiving two weeks 200 % BCAA (T2-200) even at the end of the experiment (d 35). Among the high-weight piglets the highest final body weights were realized in in the HC group. There was no significant treatment effect on feed intake or gain-to-feed ratio ($P>0.05$).

Conclusion: The later response of heavier piglets to the dietary treatments was not attributed to feed intake as indicated also by (2). A two-week provision of certain AA above the requirement seems to have long-term effects on the growth development of weaned piglets, showing possibilities to improve group homogeneity prior to the subsequent fattening phase. The different responses of the different weight categories implicate further research on individual AA-requirements.

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31.

Influence of calcium level and source, acidification, and phytase supplementation on precaecal amino acid digestibility and intestinal microbiota of broiler chickens

Einfluss von Calciumkonzentration und -quelle, Ansäuerung und Phytasezugabe auf die praecaecale Aminosäurenverdaulichkeit und Mikroorganismen im Verdauungstrakt bei Broilern

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Phytase supplementation increased precaecal digestibility (pcd) of amino acids (AA) in some but not all broiler studies and reasons for inconsistent effects are not well studied. The Ca level of the diet has been shown to influence gastrointestinal phytate degradation [1]. Acidifying diets (i.e., replacing calcium carbonate ($CaCO_3$) by calcium-formate or adding formic acid to $CaCO_3$-containing diets) might also be a relevant factor involved in pcd of AA. This study investigated whether phytase effects on pcd of AA are influenced by Ca level and acidification of the diet, and whether effects are related to changes in microbiota composition in crop and ileum digesta.

Methods: Twelve diets without addition of mineral phosphorus were formulated with 5.8 or 8.2 g Ca/kg dry matter by inclusion of $CaCO_3$, $CaCO_3$+formic acid (FA; 6 g/kg), or calcium-formate (Ca-F) and without or with phytase (1500 FTU Natuphos E/kg). Each diet was tested in 6 pens comprising 15 broiler chickens per pen. Experimental diets were provided from day 16 post-hatch. On day 21 and 22, digesta from the posterior half of the section between Meckel's Diverticulum and the end of the small intestine was collected and pooled on a pen-basis. Statistical models comprised calcium level, acidification ($CaCO_3$, $CaCO_3$+FA, or Ca-F), phytase supplementation, and interactions between these factors as fixed effects, and a random block effect. Microbial bacterial communities of crop and ileum were previously published [2] and used to determine correlations with pcd of AA. These data were also used to predict functionality and this was done with the R package Tax4Fun2, which used the KEGG hierarchy for functional assignations.

Results: The Ca level × phytase interaction was significant for pcd of all AA ($P<0.001$). Other interactions were not significant. Increasing the dietary Ca level decreased pcd of all AA ($P\leq0.005$). Phytase supplementation increased pcd of all AA ($P\leq0.006$), by an average of 2.1 percentage points (pp) at the low Ca level but by 5.0 pp at the high Ca level ($P<0.001$). Among phytase supplemented treatments, pcd of all AA was not different ($P\geq0.520$), except for a by 1.3 pp lower cysteine digestibility at the low compared to the high Ca level ($P=0.047$). The main effect acidification was significant for all AA except cysteine. Adding FA to CaCO3 increased pcd of all AA except cysteine by an average of 1.1 pp ($P\leq0.047$). Replacing CaCO3 with Ca-F increased pcd of all AA except for aspartic acid/asparagine, cysteine, and histidine ($P\leq0.035$), on average by 1.1 pp. The pH in the ileum was positively correlated with pcd of all AA ($r=0.85$-0.90, $P<0.001$). The pcd of most AA were positively correlated with Lactobacillus johnsonii and negatively correlated with Gallibacterium sp. and Streptococcus alactolyticus ($P<0.050$). Functional prediction showed that genes related to AA degradation and biosynthesis were more abundant in crop and ileum digesta when CaCO3+FA or Ca-F was used instead of CaCO3. The higher Ca level decreased the abundance of genes related to AA metabolism and biosynthesis in crop and ileum digesta. Adding phytase increased the abundance of genes related to the metabolism of some AA and decreased effects on the metabolism of other AA in both crop and ileum digesta.

Conclusions: The results suggest that dietary Ca levels, but not acidification contribute to inconsistent phytase effects on pcd of AA in literature. Acidification increased pcd of AA. Microbial fermentation up to the end of the small intestine may have contributed to changes in pcd of AA.

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32.

Effect of conjugated linoleic acid and essential fatty acid supplementation on plasma leptin and adiponectin concentrations and hepatic lipid metabolism of dairy cows during late pregnancy and early lactation

Einfluss von konjugierter Linolsäure und essentiellen Fettsäuren auf die Plasmakonzentrationen von Leptin und Adiponektin und auf den Fettstoffwechsel in der Leber bei Milchkühen während der Spätträchtigkeit und Frühlaktation

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Hepatic lipid metabolism is involved in adapting energy metabolism for milk production around calving in dairy cows and is regulated by hormones such as leptin and adiponectin (1). Supplementation of conjugated linoleic acid (CLA), especially t10, c12 CLA, improves energy status in cows by reducing milk fat release (2). Furthermore, hepatic energy metabolism might be affected by supplementation of essential fatty acids (EFA), especially α-linolenic acid (3). The objectives of the present study were to study the effects of CLA and EFA supplementation on the mRNA abundance of genes related to hepatic lipid metabolism and to test whether these effects were associated with changes in plasma concentrations of leptin and adiponectin. We hypothesized that a combined EFA and CLA treatment may alleviate hepatic energy load by affecting leptin and adiponectin response on gene expression associated with energy metabolism. **Methods:** Forty rumen cannulated German Holstein cows (11,000 kg milk/305 d in 2nd lactation) were set-up in 5 blocks of 8 cows, respectively, from wk 9 ante partum (ap) to wk 9 post partum (pp). Total mixed rations were fed ad libitum during lactation (wk 22-6 ap and wk 1-9 pp, 7.1 MJ NEL/kg dry matter (DM)) and dry period (wk 6-1 ap, 6.5 MJ NEL/kg DM). Cows were daily supplemented from wk 9 ap until wk 9 pp by an abomasal tube either with coconut oil (CTRL, 76g/d), linseed and safflower oil (EFA, 78 and 4 g/d), Lutalin® (CLA, c9, t11 and t10, c12, 10 g/d each) or EFA+CLA (2). During the dry period, each dose was halved. Plasma concentrations of leptin and adiponectin were measured in blood samples taken on d 63, 42, 35, 28, 21, 10 ap, on d 1 pp and once weekly up to d 56 pp. Liver tissue samples were obtained on d 63 and 21 ap, on d 1, 28, and 63 d pp to measure triglyceride content and mRNA abundance of acyl-CoA-synthetase, long chain 1 (ACSL1), carnitine palmitoyl-transferase 1A (CPT1A), acyl-CoA-dehydrogenase, very long chain (ACADVL), acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FAS), diacylglycerol O-acyltransferase 1 (DGAT1), microsomal triglyceride transfer protein (MTTP), fatty acid-binding protein 1 (FABP1), sterol regulatory element-binding factor 1 (SREBF1), peroxisome proliferator-activated receptor α (PPARA) and peroxisome proliferator-activated receptor γ (PPARG). Data were analysed using the MIXED procedure of SAS by repeated measurements ANOVA containing EFA, CLA, time, block and respective interactions as fixed effects, as well as calving interval and projected milk yield during 2nd lactation as covariates. **Results:** Plasma leptin and adiponectin concentrations decreased after calving ($P<0.001$) in all groups. Plasma leptin was higher ($P<0.05$) on d 10 ap in EFA than in CTRL. Plasma adiponectin was decreased by EFA treatment on d 42 and 21 ap and showed higher concentration on d 21 ap in EFA+CLA than in CTRL. Hepatic triglyceride concentration was decreased ($P<0.05$) in both CLA groups on d 28 pp. Abundance of ACSL1 mRNA was highest ($P<0.05$) in CLA cows and decreased ($P<0.05$) by EFA treatment on d 28 and 63 pp, respectively. Abundance of CPT1A and ACADVL mRNA were decreased ($P<0.05$) by EFA treatment on d 1 and 28 pp and CPT1A mRNA was increased ($P<0.05$) by CLA treatment on d 63 pp. The ACC1 and FAS mRNA abundance was decreased ($P<0.05$) by EFA treatment on d 21 ap (only FAS) and on d 63 pp. FABP1 mRNA was higher ($P<0.05$) on d 63 ap in EFA+CLA than in CLA and was decreased ($P<0.05$) by EFA treatment on d 63 pp. SREBP1 mRNA was highest ($P<0.05$) in CLA cows on d 28 pp and was decreased ($P<0.05$) by EFA treatment on d 1 and 28 pp. PPARG mRNA was increased ($P<0.05$) by EFA treatment on d 1 pp. **Conclusions:** Reduced hepatic triglyceride concentration pp in CLA-treated cows was the consequence of reduced body fat mobilisation shown by Vogel et al. (2) but might in addition result from stimulation of fatty acid oxidation by CLA treatment. EFA treatment pp indicated mostly inhibitory effects on gene transcription related to hepatic lipid metabolism. Changes in the hepatic lipid metabolism on the transcription level pp were not associated with plasma concentrations of leptin and adiponectin.

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33.

Effects of supplementation of essential fatty acids combined with conjugated linoleic acid on plasma urea, liver mRNA expression of genes related to the urea cycle and free amino acids in plasma and whey in dairy cows during the transition period

Einfluss einer Supplementierung von ungesättigten Fettsäuren kombiniert mit konjugierten Linolsäuren auf den Plasma-Harnstoff, die mRNA-Expression von Genen des Harnstoffzyklus im Lebergewebe und die freien Aminosäuren im Plasma und der Molke bei Milchkühen während der Transitperiode

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Over the last few decades, diets for dairy cows have changed dramatically. With increasing milk yield, it has become a common practice to feed TMR containing high amounts of corn silage rather than pasture-based feeding systems. Therefore, the intake of essential fatty acids (EFA) and in addition, level of rumen and tissue conjugated linoleic acid (CLA) production decreased (1). Some CLA isomers reveal metabolic effects in dairy cows, such as milk fat reduction and glucose-sparing effect. Lower milk protein and urea levels, which are possibly related to higher body protein accretion and nitrogen retention, were found following CLA supplementation (2). In a recently published study, we could confirm milk protein and urea lowering effects in CLA-treated cows whereas EFA treatment increased urea concentration in milk (3). Therefore, the aim of the present investigation was to measure plasma urea, RNA expression of genes related to the urea cycle in liver tissue and free amino acids in plasma and whey using the plasma, milk and liver tissue samples of the current study (3) to get more insights into the protein metabolism of CLA and EFA-treated cows.

Methods: Rumen fistulated German Holstein cows (n = 40; 11.000 kg milk/305 d in 2nd lactation) were studied in 5 blocks of 8 cows from week 9 before until week 9 post partum (pp). A corn silage based TMR was fed ad libitum. The cows were fitted with abomasal tubes and assigned to one of four supplementation groups: CTRL (coconut oil, 76g/d), EFA (linseed and safflower oil, 78 and 4 g), CLA (c9, t11 and c10, t12 in equal amounts, 10g/d), and EFA+CLA. The dosages were halved during dry period. Plasma samples were collected at day -63, -42, -35, -28, -21, -10, +1, +7, +14, +21, +28, +35, +42, +49 and +56. Milk samples were taken once weekly. At day -63, -21, +1, +28 and +63, liver samples were obtained by needle biopsy. The mRNA abundance of genes related to the urea cycle (ARG1, ASL, ASS, CPS1, OTC) was conducted by real-time PCR. In plasma and whey samples of day 28 pp, amino acid composition was determined using HPLC. Data were analyzed using the MIXED procedure of SAS by repeated measurement ANOVA containing fixed effects of time, treatment with EFA and CLA, block and the interaction of time, EFA and CLA. Calving interval and milk yield during 2nd lactation were used as covariates.

Results: Plasma urea was reduced by CLA and was increased by EFA treatment pp ($P<0.05$). The hepatic mRNA abundance of ARG1, ASL, ASS, CPS1 and OTC decreased in CLA-cows with ongoing lactation ($P<0.05$), and at day 63 ARG1 mRNA abundance was higher ($P<0.05$) in EFA than in EFA+CLA as well as CPS1 was higher in EFA than CLA group. Feed intake and milk production did not differ among groups on day 28 pp (3). Several amino acids in plasma (Phe, Leu, Met, His, Cys, Ser, Tyr) were lowered by CLA treatment at day 28 pp ($P < 0.05$). In whey, some amino acids (Asp, Ser, Tau, Trp) decreased and some (Arg, Cit, Cys, Ile) increased due to CLA administration. Furthermore, EFA-supplementation reduced Arg in plasma ($P < 0.05$).

Conclusions: Lower plasma urea, decrease of hepatic RNA abundance of urea cycle related genes and several lowering effects on amino acids underline the assumption that CLA-treatment is associated with higher protein accretion and nitrogen retention. Low Arg in plasma of EFA-cows fits to higher milk and plasma urea and higher RNA abundance of genes related to the urea cycle. Lower urea production of CLA-treated cows goes along with increased Arg und Cit in whey. Increased Cit in whey of CLA- and EFA+CLA-cows may point to a higher activity of iNOS in the mammary gland of CLA-treated cows.

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34.

Effects of supplementation of essential fatty acids combined with conjugated linoleic acid on mammary proteins and RNA abundance related to milk fat and protein production

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Supplementation with conjugated linoleic acids (CLA) leads to reduced milk fat content and improved energy balance in dairy cows. The mechanisms of milk fat reduction are not entirely clear. CLA inhibit de novo fatty acids synthesis in the mammary gland and additionally, uptake of fatty acids from lipoproteins (1). Effects of feeding essential fatty acids (EFA), such as α-linoleic acids, on milk fat content are varying: diet-induced drop and rise of milk fat as well as lack of changes in milk fat were reported (1). Beyond decreasing effects on milk fat content, lower milk protein and urea levels due to CLA feeding were published recently in a companion paper (2). Therefore, the objective of the present study was to identify effects of CLA- and EFA-treatment on mammary fat and protein synthesis by quantification of proteins and RNA abundance related to milk fat and protein production.

Methods: Rumen fistulated German Holstein cows (n = 40; 11,000 kg milk/305 d in 2nd lactation) were studied in 5 blocks of 8 cows from week 9 before until week 9 after parturition. A corn silage based TMR was fed ad libitum. The cows were fitted with abomasal tubes and assigned to one of 4 supplementation groups: CTRL (coconut oil, 76g/d), EFA (linseed and safflower oil, 78 and 4 g), CLA (c9, t11 and c10, t12 in equal amounts, 10g/d), and EFA+CLA. The crude fat content of the TMR was 23g per kg DM. The dosages were halved during dry period. At the end of the study, the animals were slaughtered and udder tissue was collected. Proteins of the mammary gland (ACC, FABP4, FAS, SREBP1C (68 and 120 kDa), mTOR and p-mTOR, S6K1 and p-S6K1, PPARγ) related to milk fat and protein production were determined using Westernblot. Furthermore, we quantified RNA abundances of these proteins by quantitative Real-Time Reverse-Transcription PCR. Data were analyzed using the MIXED procedure of SAS by repeated measurement ANOVA containing fixed effects of treatment with EFA and CLA, block and the interaction of EFA × CLA. Calving interval and milk yield during 2nd lactation were used as covariates.

Results: Effects on milk composition and production of the cows in the present study have been published recently (2): Beyond lower levels of milk fat and protein as well as milk urea, milk yield remained unaffected. FAS was reduced due to CLA-treatment ($P < 0.01$), whereas FABP4 was increased in CLA-cows ($P < 0.05$). The active form of SREBP1C (68 kDa) was lower in EFA-treated cows ($P < 0.05$). However, CLA-supplementation tended to decrease SREBP1C (68 kDa; $P = 0.06$) as well. In CLA-cows, S6K1 was increased in udder tissue ($P < 0.05$). ACC, SREBP1C 120 kDa, mTOR, p-mTOR, p-S6K1 and PPARγ were not significantly affected by EFA or CLA-treatment. RNA abundance of ACC, FAS and SREBP1 was lower due to CLA-feeding. The RNA expression of the other determined factors related to milk fat and protein synthesis remained unaffected by CLA- or EFA supplementation.

Conclusions: Effects of CLA supplementation on milk fat and protein synthesis are regulated on RNA and protein base. According to the present results, milk fat reduction of CLA treatment is mainly related to reduction of RNA abundance of FAS, which was also reflected by lower protein levels of FAS. For all other factors, mRNA abundance and protein levels were not consistent. Mainly EFA, but also CLA supplementation lowered the activation of SREBP, pointing to effects of EFA as well as CLA on glucose utilization and fatty acid synthesis. In accordance with a study measuring proteins of skeletal muscle tissue after CLA-supplementation (3), we recorded an increase of S6K1 due to CLA-feeding, thus, reflecting CLA-effects on protein metabolism.

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35.

Effects of maternal conjugated linoleic acid and essential fatty acid supply on the intestinal microbiota of calves

Einfluss einer maternalen Supplementierung mit konjugierter Linolsäure und essentiellen Fettsäuren auf die intestinale Mikrobiota von Kälbern

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The gut microbiota is known to be able to influence the host´s energy metabolism, inflammation and immunity, which in turn demonstrates the importance of the development of the early microbiota in neonates. Conjugated linoleic acid (CLA) isomers were shown to have direct effects on the gut microbiota in mice (1). Furthermore, CLA isomers are known to alter the fatty acid composition and inter alia the content of polyunsaturated fatty acids in cow´s colostrum and milk (2), which are the most important nutrient sources of the calf after birth. Especially omega-3 polyunsaturated fatty acids are discussed to establish the eubiosis in the gut (3), which is crucial for the maintenance of gut health. The present study investigated the effects of a maternal CLA supplementation with and without combined essential fatty acids (EFA) on the intestinal microbiota of 5-day old calves.

Methods: Nine Holstein cows were abomasally supplied either with coconut oil (control, CON, 76 g/d), CLA (Lutalin™ = feed additive, 25 % cis-9, trans-11 CLA + 25 % trans-10, cis-12 CLA, 38 g/d) or a combination of CLA+EFA (38 g/d Lutalin™ + 78 g/d linseed oil + 4 g/d safflower oil) from week 9 before calving and during the following lactation period (2). The 9 calves (4 male calves, 5 female calves) born from mentioned dams and fed with their milk were slaughtered on day 5 after birth. Intestinal content from mid jejunum was collected and microbial DNA was isolated by using the QIAmp® DNA Stool Kit. Analyses based on the targeted sequencing of the microbial 16S rRNA gene were conducted. The operational taxonomic units clustering and the taxonomic assignment were performed using mothur 1.44.1 and the Silva database (release 138). Statistical analyses on phylum level were done in R.

Results: Proteobacteria (53.4 %), Firmicutes (32.9 %) and Bacteroidota (8.9 %) were the major representatives in the jejunal content of the examined calves. The relative abundance of Cyanobacteria and Euryarchaeota was decreased in CLA+EFA calves compared to CON and CLA calves ($P < 0.05$). In contrast, the relative abundance of Chloroflexi bacteria was increased in the CLA+EFA group compared to the other groups ($P < 0.05$). Influences on the abundance of Firmicutes were not detected ($P > 0.05$).

Conclusion: The maternal supplementation with CLA seemed to have only marginal effects on the jejunal microbiota of calves, which is probably related to the low CLA effects on the milk fatty acid composition in this early period after calving as demonstrated by the fatty acid concentrations in plasma of calves (2). However, a maternal supplementation of a combination of CLA+EFA resulted in marked alterations in the neonatal jejunal microbiota indicated by shifts in the abundance of the detected different phyla. Therefore, maternal EFA supplementation had an major impact on the neonatal intestinal microbiota in the early state of life.

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36.

Drinking behavior as an indicator for the drinking water quality in dairy cows

Trinkverhalten als Indikator für die Tränkwasserqualität bei Milchkühen

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An adequate water supply is necessary for optimal feed consumption, productivity, health and animal welfare. Impaired water quality due to long trough cleaning intervals might affect behavior and welfare of animals. The aim of this study was to investigate to what extent neglecting the cleaning of water troughs is reflected in the drinking behavior of dairy cows on farms.

Methods: This study was conducted at a commercial dairy farm with a herd of 120 lactating cows that were held in a symmetrical free-range barn, milked with an automatic milking system, and fed once a day in the morning. The drinking behavior at two identical open troughs (2.0 m length, 0.43 m wide, 0.15 m depth, 70 L volume) and two identical double valve troughs (0.73 m length, 0.32 m wide, 0.10 m depth, variable volume) made of stainless steel was recorded daily by video in the first 2 h after feeding. The study was done over a period of 15 d in the beginning of December 2019 and was repeated in February of 2020. The two different troughs (one of each trough design) was cleaned with a brush and drained on daily basis and the other two were not cleaned at all following a 2 × 2 Latin square study design, which enables the animals to choose between cleaned and uncleaned troughs. Each drinking process was analyzed and characterized using Behavioral Observation Research Interactive Software (BORIS; Friard and Gamba, 2016). Water was characterized on daily basis by ATP water hygiene tests (3M, Neuss, Germany), water temperature, pH-value, and visual assessments. At the beginning and at the end of each study period physico-chemical and microbiological water quality was evaluated. Furthermore, ambient temperature and ambient humidity were measured for each trough throughout the study periods. To address effects besides trough design such as the trough position, the troughs (n = 4) were analyzed individually. Data was analyzed using a mixed model with cleaning scheme and trough as fixed factors as well as Spearman rank correlations in SAS (SAS 9.4).

Results: ATP-value of the drinking water was hardly influenced by the cleaning scheme. Furthermore, physico-chemical, and microbiological water quality indicators were acceptable in the beginning and the end of each study period regardless of the cleaning scheme. The cleaning scheme alone failed to be mirrored in drinking behavior. However, significant behavioral alterations were observed for the interaction of cleaning scheme × trough. At one of the valve troughs, a higher number of drinking processes were observed while cleaned daily than without cleaning ($p < 0.0001$). In the cleaned condition, the duration of active water intake decreased at one of the open troughs ($p < 0.05$), while the duration of tasting behavior increased ($p < 0.05$). The number of antagonistic behaviors doubled for the most frequently used open trough with daily cleaning compared to no cleaning ($p < 0.001$). The increased number of drinking processes at uncleaned troughs might be caused by displacements from cleaned troughs due to antagonistic behavior. The drinking water ATP value correlated negatively with the duration of the drinking processes ($R = -0.23$, $p < 0.05$) and positively with the duration of active water uptake ($R = 0.20$, $p < 0.05$) and the number of antagonistic behaviors ($R = 0.22$, $p < 0.05$).

Conclusion: Despite a low-risk scenario, caused by stainless steel troughs and low ambient temperatures, which resulted in a high biological quality of the drinking water, alterations in drinking behavior were observed. The duration of drinking processes and active water intake as well as the number of antagonistic behaviors at troughs might be useful behavioral indicators for livestock drinking water quality. However, daily measurement of physico-chemical and microbiological water quality would be preferable in future studies.

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37.

Trough design influences drinking behavior of dairy cows

Das Tränksystem beeinflusst das Trinkverhalten von Milchkühen

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Water supply is an essential factor to maintain performance, health and well-being in dairy cows. The troughs used to provide water on dairy farms range from high volume, open troughs, over valve troughs to small volume bowl drinker. However, comparisons of different trough designs are often based on the amount of water consumed and thereby neglecting effects on the drinking behavior. Therefore, the aim of this study was to compare two different trough designs in regard to the drinking behavior of dairy cows.

Methods: This study was conducted at a commercial dairy farm with a herd of 120 lactating cows that were held in a symmetrical free-range barn, milked with an automatic milking system, and fed once a day in the morning. Water was offered with two fixed installed identical open troughs (2.0 m length, 0.43 m wide, 0.15 m depth; 70 L volume) and two identical double valve troughs (0.73 m length, 0.32 m wide, 0.10 m depth; variable volume) made of stainless steel. The drinking behavior at all troughs present in the barn was recorded daily and simultaneously by video in the first 2 h after feeding. The study was conducted over a period of 15 d in the beginning of December 2019 and was repeated in February of 2020. The physico-chemical and microbiological water quality at the beginning and the end of the study was similar between the two trough types and below the recommended thresholds for livestock drinking water. Each drinking process was characterized using Behavioral Observation Research Interactive Software (BORIS; Friard and Gamba, 2016) for counting the total numbers of 'drinking events', 'swallows per drinking events', 'tasting behaviors', 'swallowing difficulties', 'antagonistic behaviors', 'drinking breaks' and 'breaking off the drinking event due to antagonistic behavior' as well as the duration of 'total drinking event', 'tasting behavior', 'drinking breaks' and 'active water intake'. The recorded behavioral parameters were analyzed using a linear model with post hoc Tukey correction and trough design as the fixed factor in SAS (SAS 9.4).

Results: More drinking events were registered at open troughs (n = 2,435) than at the valve troughs (n = 1,650). Duration of the drinking event was shorter ($p < 0.0001$) at open troughs than at valve troughs. Furthermore, the observed cows tended to show more antagonistic behavior at open troughs compared to valve troughs ($p = 0.1$), leading to a higher proportion of breaking off drinking events due to antagonistic behaviors ($p < 0.0001$) at open troughs. The drinking events at valve troughs showed a higher number of drinking breaks ($p < 0.0001$) and swallowing difficulties ($p < 0.0001$) compared to open troughs. Recorded dairy cows smelled more often at open troughs before initiating the drinking event than at valve troughs ($p < 0.01$), but tended to show longer total tasting behavior at valve troughs compared to open troughs ($p = 0.1$). The duration of drinking breaks and duration of active water intake and the number of swallows per drinking event was not affected by trough design.

Conclusion: This study indicates that dairy cows drinking behavior is influenced by the troughs design. Cows seem to prefer open troughs over valve troughs as they visited those more often and showed fewer drinking breaks and impairments of the drinking events. Unfortunately, trough design could not be distinguished from position effects. Further research on the effects on dairy cows drinking behavior such as trough position or trough environment might be useful to optimize water provision on dairy farms and thereby improve animal welfare.

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38.

Does the temperature-humidity index (THI) allow conclusions to be drawn about the water requirements of pigs?

Lässt der Temperatur-Luftfechtigkeits-Index Index (THI) Rückschlüsse auf den Wasserbedarf von Schweinen zu?

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Water is the basis of existence for every living organism and thus also for the pig. The calculated water requirement for fattening pigs is 100 ml/kg/day live weight (1). However, water requirement is influenced not only by life weight, but also by environmental conditions such as, climate. In order to determine the water requirement depending on the climate in the house, the temperature-humidity index (THI) was used as a parameter for the climate in the house. The THI combines the climatic influencing factors temperature and relative humidity in one index and is frequently used as an indicator for heat stress and well-being. An optimal housing climate for the pigs is particularly important for successful animal husbandry, especially with regard to longer periods of heat which cannot completely counterbalanced by technical means (e. g. ventilation). While the THI is often used in the cattle sector, it is hardly considered in the fattening pig sector. Despite closed housing buildings in conventional pig farming, the influence of the weather also plays an important role with regard to the climate in the barn. The aim of the present study was to examine whether the THI is a good indicator for sufficient water supply and thus its relevance in fattening pig production.

Methods: Eighty fattening barrows (live weight 24.3 ± 4.3 kg) were randomly allocated to 4 pens (20 pigs/pen) in the same pig unit and subjected to a 3-phased fattening regimen with starter, grower and finisher phase consisting of 4 weeks each. Data collection was confined to starter and grower phase (02 July 2020 to 20 August 2020). Feed was provided ad libitum via dry feeders with an animal-to-feeder space ratio set at 4:1. Two nipple drinkers were installed per pen, with a flow rate adapted to the weight (animal-drinker ratio 10:1). Daily water consumption was recorded for each pen using water meters on the nipple drinker. Once a week the pigs were weighed. From the average weights, the daily water requirement was derived based on the water requirement of 100 ml/kg BW/day.
Data loggers of Driesen + Kern rugged HumiLog were placed in the middle of each pen and outside of the barn. Ambient temperature (T, in °C) and relative humidity (RH, in %) were continuously recorded at minute intervals and THI was calculated with the inside values based on the equation (2):
THI = [(1.8 x T) + 32] - [0.55 x (RH / 100)] x [((1.8 x T) + 32) - 58]
The data was evaluated on a daily basis, so that the average value of the THI was calculated for the individual days. Furthermore, the deviation of the daily water consumption from the calculated water demand was determined.

Results: The average outside temperature during the test period was 21.83 +/- 6.26 °C (min.: 9.82 °C, max.: 46.45 °C). The average relative humidity was 65.40 +/- 19.62 % (min.: 19.35 %, max.: 99.90 %). The average THI over all 4 pens were 70.58 +/- 3.47 (min.: 63.50, max.: 83.00). The average THI per day in all 4 pens tended to increase during the test period with partly substantial fluctuations. Water consumption was subject to similar fluctuations as the THI, with a rising trend over the trial period.
All pens had a higher water consumption than the calculated demand during the entire duration of the test, with the exception of 3 periods for pen 3 and 4 periods for pen 4 (mean value deviation pen 1: 82 %; pen 2: 74 %; pen 3: 27 %; pen 4: 47 %). Water consumption correlated significantly positively with the THI in all pens ($p \leq 0{,}05$) (pen 1: rank correlation coefficient spearman (rs) = 0.65; pen 2: rs = 0.76; pen 3: rs = 0.51; pen 4: rs = 0.70).

Conclusion: It could be shown that the calculated water demand would not have covered the actual water demand of the pigs in the present study. Furthermore, a close correlation between the THI and the water consumption was demonstrated, which indicates that the THI can provide information about the pigs' water requirements. Therefore, it should be considered to use the THI also in fattening pigs as an indicator for an adapted indoor climate.

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39.

Impact of dried food residues in a diet on the fecal microbiota of cats

Einfluss von getrockneten Speiseresten in einem Alleinfutter auf die fäkale Mikrobiota von Katzen

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The use of food residues for animal nutrition is of increasing interest, since it may imply both economic and ecological advantages (1). Although legal restrictions currently limit the use of food waste as a feed ingredient, strategies to reduce food waste are promoted, and might also contribute to policy changes. The present study aimed to evaluate the effects of dried food residues (DFR) from hotel catering in a diet on the intestinal microbiota of cats.

Methods: Seven adult cats received a complete diet with or without DFR (0, 5, 10 or 15 %). All the cats were fed the same experimental diet at the same time, where the amount of DFR in the diet increased in each subsequent feeding period. The cats were fed individually, and the remainder was weighed back daily. The diets were based on ground beef, rice flour, rapeseed oil, cellulose and mineral and vitamin supplements. The analyzed nutrient composition of the DFR was: dry matter (DM) 91.2 g/100 g; crude ash 5.97 g/100 g DM, crude protein 25.9 g/100 g DM, crude fat 24.7 g/100 g DM, crude fiber 3.46 g/100 g DM. The analyzed crude protein (41.5-42.3 g/100 g DM) and calculated metabolizable energy (ME) concentrations (2.00-2.02 MJ/100 g DM) were comparable among the four diets. Fecal samples were collected at the end of each three-week feeding period. Microbial metabolites in the feces were analyzed using standard laboratory methods, and the fecal microbiota by 16S rDNA sequencing. For statistical data analysis, SPSS 22 was used (GLM repeated measures, calculation of polynomial contrasts, level of significance: $\alpha = 0.05$).

Results: No group differences in feed intake (g DM/kg body weight (BW)/day) and BW of the cats could be observed ($P > 0.05$). The dietary inclusion of DFR increased the diversity of the fecal microbiota of the cats, as demonstrated by an increase of the richness and the evenness and Shannon indices, and a decrease of the Simpson index (linear contrasts: $P < 0.05$). In addition, an increase of the relative abundance of the bacterial order Coriobacteriales and the bacterial genera Collinsella, Lachnoclostridium, Libanicoccus and Romboutsia (linear contrasts: $P < 0.05$) was observed. The fecal propionic acid concentrations increased from 27.9 ± 15.5 µmol/g (0 % DFR) to 45.8 ± 19.8 µmol/g (15 % DFR), and the fecal n-valeric acid concentrations from 6.42 ± 3.13 µmol/g (0 % DFR) to 9.19 ± 4.54 µmol/g (15 % DFR) (linear contrasts: $P < 0.05$). Fecal ammonium concentrations were not affected by the dietary inclusion of DFR.

Conclusions: The observed changes in the composition and metabolic activity of the fecal microbiota indicate that especially undigestible carbohydrates, derived from the DFR, were microbially fermented in the intestine of the cats. Although the intestinal microbiota of cats is able to ferment dietary fiber (2), lower amounts of DFR in a diet might be preferable in order to prevent major shifts of the balanced gut microbiome.

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40.

Investigations about the optimal crude protein concentration in diets for the yellow mealworm (*Tenebrio molitor*)

Untersuchungen zum optimalen Rohproteingehalt in Futterrationen für den gelben Mehlwurm (Tenebrio molitor)

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Introduction: Insects are discussed as efficient producers of high-quality food and feed protein and hence as novel kind of feed transformers potentially mitigating environmental impacts of common livestock production systems. Although intensive research about insects for food and feed application has been conducted in recent years, feed requirements of insects did not receive the desired attention. Especially for *Tenebrio molitor*, information about nutrition requirements is limited. The present study aimed to determine the optimum crude protein (CP) requirement of growing Tenebrio molitor.

Methods: Fifty thousand small growing mealworms (average bodyweight of 40 mg) were allocated randomly to fifty groups (approx. 1000 animals per group) and were housed in plastic bowls at 28 °C and 50 % relative humidity in a climate cupboard. Nine isoenergetic experimental diets with varying CP concentration (g CP per 100 g dry matter (DM): 9.0, 11.5, 13.9, 16.3, 18.6, 20.6, 23.0, 25.7, 28.0) were formulated on base of wheat flour, starch, gluten, and a vitamin-mineral mixture without added pure amino acids. A commercial feed for laying hens (22.7 g CP per 100 g DM) served as control since commercial mealworms are often produced with this type of feed. Each five mealworm groups were allocated to the ten diets (nine experimental, one control). Feed supply (FS) to each animal group was adjusted daily according to visual judgement of complete feed consumption in order to estimate ad libitum feed intake. Biomass (BM) of mealworms and feed supply (FS) were recorded per bowl every five days starting with experimental day 10. On day 31, mealworms were euthanized by freezing at -21 °C. Weight gain (WG), final BM, and total FS per mealworm group were statistically analysed using ANOVA (all ten feed variants) as well as regression analysis (nine experimental feed variants) using dietary CP concentration as regression determinant.

Results: Total FS, final BM, and WG responded to rising dietary CP concentrations according to a cubic function (R^2=0.99) with increasing values at very low dietary CP and maxima at around 12.0 g CP per 100 g DM, followed by a decrease at further rising CP concentrations ($p<0.01$). Zootechnical responses of the control feed (22.7 g CP per 100 g DM) were equal to animals fed the experimental diet containing 11.5 g CP per 100 g DM

Conclusions: Interestingly, mealworms fed with experimental diets containing 12 g CP per 100 g DM of putatively low protein quality (predominantly gluten) performed equal to commercial layer feed. This gives rise to the hypothesis that CP requirements of mealworms are significantly lower than currently assumed. The depressive effects of experimental diets with high CP contents might have been caused from physiological reasons (e.g. amino acid imbalances) as well as physical problems (e.g. sticky consistency of feeds). In total, protein requirements of insects in terms of dietary concentrations as well as amino acid composition need to be investigated more in detail. Conclusions by analogy from common livestock species should be done cautiously.

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41.

The impact of adaptations in the diet on the occurrence of symptoms in horses diagnosed with polysaccharide storage myopathy 2: a case report

Der Einfluss einer Anpassung der Fütterung auf die Ausprägung der Symptome in Pferden mit diagnostizierter Polysaccharid Speicher Myopathie 2: ein Fallbericht

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Polysaccharide storage myopathy 2 (PSSM2) is an inheritable disease affecting horses of various breeds, gender and age [1]. It is associated with a reduction in wellbeing and performance. A large symptomatic outbreak was observed in horses in Berlin-Brandenburg, Germany and poor hay quality was suspected to be the trigger for the observed symptoms such as muscle loss, changes in behaviour (aggressiveness, numbness) and poor performance. **Methods:** Genetic testing from hair samples was performed for PSSM2 and the subgroups P2 (myotilin), P3 (filamin C), P4 (myocenin) and Px (Ca^{2+} ion channel) in 106 symptomatic and 20 asymptomatic horses in Berlin-Brandenburg in 2019-2020 (Generatio GmbH and Center for Animal Genetics, Tübingen, Germany). Random samples revealed that these horses often received hay or haylage of inadequate quality and many of the horses did not receive balanced rations. The owners of symptomatic horses tested positive were offered to participate in a study investigating the impact of diet on PSSM2 symptoms.These horses received a starch restricted diet high in protein and fat based on hay, pelleted sainfoin (14.5 % crude protein), and linseed oil. Additionally, a feed additive high in vitamin E and selenium was offered and the diet ballanced for the remaining minerals and vitamins. The horses were exercised regularly. Changes in wellbeing, performance and body condition were documented. **Results:** Out of the tested animals, 89 horses (71 %) proved positive for at least one variant. The most commonly detected PSSM2 variation was p2 (40 %) followed by px (34 %), p4 (14 %) and p3 (12 %). In these horses, the variants were observed single (59 %), in duplicates (34 %) or triplicates (7 %). A wide variety of symptoms was associated with the disease, affecting behaviour, the locomotor system, performance, the gastrointestinal tract, immune system, and neuromuscular system. Additionally, the skin, respiratory and cardiovascular system as well as the urogenital tract showed symptoms in some cases. Commonly, the patients showed signs of discomfort and pain, tried to avoid work, and could even turn aggressive towards companions and caretakers because of the discomfort. Due to disease associated complications, two horses had to be euthanized. Changes in the diet were performed in 51 horses. Significant improvement was observed regarding performance, behaviour, and body condition. Furthermore, horses suffering from skin and/or coat problems or faecal water recovered. However, some symptoms, such as muscle bumps or respiratory sound, were not affected. This might explain the improvement of some symptoms. **Conclusions:** Balancing the diet by reducing soluble carbohydrates, increasing protein and fat content, and adding vitamin E and selenium among others might help to reduce symptoms in PSSM2 positive horses. However, targeted research is needed to identify the regulating variables as well as suitable inclusion levels.

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42.

The effect of pre-weaning supplemental liquid feeding on piglet survival, growth, sows body condition and milk composition

Einfluss einer ergänzenden Flüssigfütterung vor dem Absetzen auf Überleben und Wachstum der Ferkel, der Körperkondition der Sauen und die Milchzusammensetzung

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Increased sow prolificacy has resulted in the reduced availability of maternal milk for each piglet (1). Supplemental feeding strategies (e.g., creep feeding and additional milk feeding) have been shown to benefit piglet growth, while the beneficial effects in previous studies are not consistent and supplemental liquid feeding (LF) starting from early postnatal stage might be more effective than dry feeding (DF) (2). The aim of this study was to investigate the effect of LF during the suckling period on piglet growth [pre- (PRW) and post-weaning], sow body condition (BC) and milk composition (MC), compared with DF. **Methods:** In total, 35 DanBred sows (parities 1-5) and their litters were randomly assigned to LF (n = 17) or DF (n = 18) group. Piglets in the LF group (n = 226) received additional milk-based replacer (1-15 d) followed by a plant-based liquid feed (16-27 d), while DF piglets (n = 205) received additional plant/whey-based dry feed (6-21 d). Piglets in LF and DF groups received the same weaning (22-27 d) and post-weaning (28-69 d) feeds. We measured piglet body weight (BW) daily from 0-26, and at 30, 44 and 69 d; sow BC (BW, back-fat thickness and body condition score) at 7 d antepartum and 26 d postpartum, and MC at 1, 7 and 21 d of lactation. Data was analysed using the MIXED procedure of SAS. Differences among least square means were considered significant at $P < 0.05$. **Results:** The LF piglets had lower PRW mortality (17 ± 2.8 vs. 24.5 ± 2.6%; $P < 0.05$), higher BW (44 d: 11 ± 0.2 vs. 9.9 ± 0.2 kg; $P < 0.01$; 69 d: 24.3 ± 0.2 vs. 22.3 ± 0.2 kg; $P < 0.01$) and average daily gain (ADG 26-69 d: 0.41 ± 0.01 vs. 0.37 ± 0.01 kg/d; $P < 0.05$) than DF piglets. Male LF piglets tended ($P < 0.1$) to be heavier at 18 and 20-25 d, and were heavier ($P < 0.05$) at 30, 44, and 69 d, compared with DF male piglets. Female LF piglets were heavier ($P < 0.01$) than DF female piglets at 44 and 69 d. The ADG (26-69 d) of male and female piglets was higher ($P < 0.1$ and $P < 0.05$, respectively) in the LF group compared with the DF group. In the LF group, male piglets tended to be heavier ($P < 0.1$) at 12-15, 17 and 20-26 d, and were heavier ($P < 0.05$) than female piglets at 30 and 69 d. In the DF group, male piglets had higher (22.8 ± 0.2 vs. 21.9 ± 0.2 kg; $P < 0.01$) BW than female piglets at 69 d. When considering sow parity, in parities 1-3, LF piglets had higher BW (16-17 d, $P < 0.1$; 18-69 d, $P < 0.05$) and ADG (1-26 d: 0.21 ± 0.01 vs. 0.17 ± 0.01 kg/d; $P < 0.1$) than the DF group. Additionally, LF sow tended to have lower (4.0 ± 0.2 vs. 4.7 ± 0.2%, $P < 0.1$) milk lactose concentration than DF sows at 21 d of lactation, and sow BC did not differ between LF and DF sows at 7 d antepartum and 26 d postpartum. **Conclusion:** The LF regime improved piglet survival and growth and this effect was greater in male piglets and the offspring of sows with parities 1-3. The LF regimen did not affect sow BC or had a considerable influence on MC.

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43.

Investigation on the impact of two different fibre sources on immunoglobulin levels in sow serum and colostrum and in piglet faeces

Eine Untersuchung zum Einfluss zweier Faserquellen auf die Konzentrationen von Immunoglobulinen im Kolostrum und Serum von Sauen und im Ferkelkot

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The nutrition of sows has an impact on the composition of their colostrum and milk and may thereby influence the health and development of their offspring. Colostrum immunoglobulins are crucial for thetransfer of passive immunity in neonatal piglets [1]. Feeding different fibre sources may increase imunnoglobulins in the serum of sows and their offspring [2]. The aim of this study was to evaluate the impact of sugar beet pulp and lignocellulose on immunoglobulin titres in colostrum and in faeces of suckling piglets.

Methods: Twenty sows were randomly allocated to two different feeding groups either high in sugar beet pulp (SBP, n=10) or lignocellulose (LNC, n=10), both diets being iso-caloric and iso-nitrogenic, during gestation and lactation. Crude fibre content was 66.1 and 110.5 g/kg DM in gestation diet and 70.3 and 123.7 g/kg DM in the lactation diet for SBP and LNC respctively. The sows were housed individually, and piglets were weaned at the age of 4 weeks. Weaned piglets were reared in groups of four animals. Serum and colostrum were obtained from sows directly after birth. Fecal samples from two piglets per sow were collected on day 2, 6, 10, 14, 21, 35 and 42 after birth and at weaning. The immunoglobulin levels in serum, colostrum and faeces were measured using commercial ELISA kits. Statistical differences ($p \leq 0.05$) were calculated using Mann-Whitney-U and Friedman test. The animal trial was approved by the Regional Office for Health and Social Affairs (LaGeSo Reg. G0112/19).

Results: No significant differences between treatments were observed for immunoglobulins in colostrum (IgA, IgG, IgM) and serum (IgA, IgM) of the sows. In colostrum, the highest immunoglobulin fraction was represented by IgG, followed by IgA and IgM. Faecal Ig titres were the highest during the first week after birth and decreased as the piglets aged. The concentration of IgM were significantly lower in the faeces of 6-day-old piglets from sows fed SBP, as compared to LNC. On the other hand, and 21-day old piglets from sows fed SBP vs. LNC had significantly higher titres of IgM in their faeces.

Conclusions: Sow diets enriched with either SBP or LNC during gestation and lactation have a differential impact on the immunoglobulin level in their offspring. This may be relevant for the early immune programming and protection against antigens in the offspring.

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44.

Colostrum composition in primiparous and multiparous sows fed different dietary fibres

Kolostrumzusammensetzung bei primiparen und multiparen Sauen, die mit unterschiedlichen faserreichen Futtermitteln gefüttert wurden

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Colostrum is an important source of nutrients and bioactive compounds to newborn piglets. Dietary fibre has been reported to modify colostrum and milk composition in sows [1]. In addition, there are contradictory data on the colostrum composition in primiparous and multiparous sows [2, 3]. The aim of this study was to analyse whether colostrum composition may be affected by dietary fibre sources fed to primiparous and multiparous sows during gestation and lactation.

Methods: Twenty sows were fed experimental gestation and lactation diets enriched with either sugar beet pulp (SBP; inclusion rate: 15 % sugar beet pulp, 3 % lignocellulose; n=7 primiparous vs. n=3 multiparous sows) or lignocellulose (LNC; inclusion rate: 15 % lignocellulose, 3 % sugar beet pulp; n=7 primiparous vs. n=3 multiparous sows). The diets were iso-caloric and iso-nitrogenic and were based on barley, wheat and soybean meal. Gestating sows were kept in groups and housed individually one-week ante-partum until weaning around day 28. The sows were fed restrictively during gestation, while ad libitum during lactation. Colostrum from randomly chosen teats was collected within 10 hours after beginning of the farrowing and once the afterbirth was excreted, and was assessed for crude protein and crude fat (Weende standard procedures), lactose (enzymatic assay), immunoglobulins (ELISA), amino acids and urea (HPLC), and biogenic amines (ion-exchange chromatography). Data were analysed by Mann-Whitney U test and Spearman's rho correlations (significance at $p \leq 0.05$) (SPSS version 24.0.0.0). The animal trial was approved by the Regional Office for Health and Social Affairs (LAGeSo Reg. G0112/19).

Results: The diets showed a trend for reduced colostrum crude protein in the LNC diet (194.0 g/kg vs. 176.6 g/kg, p=0.079), but no differences were observed for crude fat (38.8 g/kg vs. 46.1 k/kg, p=0.408) or urea (1.9 µmol/L vs. 2.0 µmol/L, p=0.182). Lactose content differed significantly between the sows fed SBP and LNC (22.1 mg/g vs. 25.6 mg/g, p=0.031. Total immunoglobulin content (150.4 mg/mL vs. 129.4 mg/mL, p=0.165) and total biogenic amines (6.9 µmol/L vs. 9.6 µmol/L, p=0.968) in colostrum were not affected by the amount of fibre in the diet. Comparison of primiparous and multiparous sows independent of the amount of fibre used did not yield any significant differences in concentrations of nutrients (crude protein: 185.0 g/kg vs. 184.6 g/kg, p=0.898, crude fat: 43.6 g/kg vs. 41.4 k/kg, p=0.892, lactose: 24.6 mg/g vs. 22.6 mg/g, p=1.000), urea (2.1 µmol/L vs. 2.5 µmol/L, p=0.467) total immunoglobulins (134.6 mg/mL vs. 152.3 mg/mL, p=0.239) or total biogenic amines (8.4 µmol/L vs. 7.9 µmol/L, p=0.831).

Conclusions: We demonstrate that either high- or low-fermentable dietary fibres in gestation and lactation diets have little impact on colostrum composition in sows. Independent of the diet, primiparous and multiparous sows share similar colostrum composition profiles.

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45.

Does a high-energy diet with or without Spirulina supplementation affect body weight and blood markers of pregnant sows and their piglets?

Beeinflusst eine Hochenergiediät mit oder ohne Spirulinazulage das Körpergewicht und Blutmarker von tragenden Sauen und ihren Ferkeln?

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Pregnancy and lactation are key stages in the life of expecting mothers and newborns when adequate nutrition is of major importance (1). In this respect, an energy dense ('Western') diet rich in fat and sugars may have direct and intergenerational detrimental effects by increasing the risk for the development of obesity and related metabolic disorders. The microalga Arthrospira platensis (Spirulina) contains various bioactive compounds (2), which might potentially mediate direct and intergenerational beneficial metabolic effects. The aim of this study was to investigate if maternal Spirulina supplementation can ameliorate the detrimental effects of a Western diet in both gestating mothers as well as offspring. Pigs were used as a model organism; this also for human nutrition.
Methods: Nineteen sows (Landrace × Large White, 5 months of age) with an initial body weight (BW) of 135±0.5 kg (mean±SEM) were randomly assigned to two BW-matched dietary groups. While the control diet (CTR), rich in starch, was based on commercial recommendations for gestating and lactating pigs, the experimental diet (WES) contained high proportions of lipids (15% hydrogenated plant oil), sugars (15% fructose, 20% sucrose) and cholesterol (0.2%). The diets were isonitrogenous. The sows were kept in pairs with ad libitum access to feed and water. Daily feed intake was restricted during the following gestation days: 2 kg on days 42–95 and 3 kg on days 96–107. Part of each diet group was supplemented with Spirulina (Sp, 20 g/day), thus resulting in four experimental groups: CTR-Sp (n=5), CTR+Sp (n=5), WES-Sp (n=5), WES+Sp (n=4). Sows were artificially inseminated with semen from the same boar to minimize a genetic influence. The BW was measured at the beginning and end of the gestation period. Feed intake was recorded weekly. Data on gestation length, litter size and piglet birth weight were collected. Plasma from sows was collected shortly before insemination and before farrowing. At farrowing, blood samples were collected from the umbilical cords of the piglets. The plasma concentrations of triglycerides (TG) and total cholesterol (TC) were quantified using commercial kits. Data were evaluated (R studio, version 1.2.5042) by a Linear Mixed Model using diet, +/- Sp, piglets' gender (for piglet data only) and their interactions as fixed effects, and sow and litter size as random effects.
Results: Feed intake did not differ between groups, whereas energy intake was higher ($p<0.01$) in WES compared to CTR sows. At farrowing, the BW of the WES sows was lower than that of the CTR sows (280 vs 267±6.6 kg; $p<0.05$). The Sp supplementation did not affect feed and energy intake and farrowing BW. Furthermore, diet and Sp did not affect litter size (13.1±0.96) and pregnancy length (116±0.5 days). Birth weight was not affected by either diet or Sp. However, newborn males were heavier than females (1.65 vs 1.58±0.342 kg; $p<0.01$). Concentrations of TG and TC in sow plasma did not differ at any given time point but TG were lower in neonatal piglet plasma in both Sp groups than in the non-SP groups (18 vs 28±3.3 mg/dl; $p<0.05$). In addition, WES+Sp piglets tended to have a lower plasma TC concentration than WES piglets (33 vs 50±4.5 mg/dl; $p=0.057$).
Conclusion: These results indicate that, despite a higher energy intake, the WES sows showed a lower BW gain compared to CTR sows. While there was no direct effect of the maternal diet on plasma lipids, maternal +Sp, regardless of maternal diet, led to a reduction in plasma lipid concentration in the offspring. To further understand these effects, liver and muscle transcriptomes of sows and piglets are currently under investigation.
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46.

Effects of a high proportion of wheat or rye in compound feed under the conditions of an experimental infection with *Salmonella Typhimurium* in young pigs

Auswirkungen eines hohen Anteils von Weizen oder Roggen in Mischfutter unter den Bedingungen einer experimentellen Infektion mit Salmonella Typhimurium bei jungen Schweinen

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Salmonella in pigs is a major concern for human foodborne salmonellosis. Reducing Salmonella contamination at the farm level plays a key role in the pig production chain. Nowadays rye is acceptable for feeding pigs, the risk of ergot contamination has been significantly reduced by new hybrid varieties (1). Intensive studies on rye focused on the use of high proportions of rye in the ration with high dietary fiber contents, which acts as a substrate for microorganisms in the hind gut (colon). This helps to raise the production of short chain fatty acids and therefore leads also to the formation of butyrate which further promotes intestinal health and could reduce invasive Salmonella infections (2,3). The focus of interest was whether the effects of a high rye content in the feed mixture of young pigs, could reduce the prevalence of Salmonella in fecal shedding. In addition, the issue of animal health on growing performance was also of great concern.

Methods: Forty-two piglets (25 d of age and initially 7.5 kg) were distributed among two diet groups and fed either a diet containing 69% wheat (control group) or 69% rye (experimental group) for a 28 days infection period to evaluate the effects of a high proportion of rye on reducing *Salmonella Typhimurium*. After one-week adaptation period, all piglets were orally challenged with a 2mL dose of 107 CFU/mL of ST. Salmonella in fecal shedding were evaluated at day 1, 3, 5, 7 and then weekly after infection. At the end of the experimental period (at day 28 after infection), the piglets were euthanized to sample feces and cecal digesta contents to determine the bacterial counts of Salmonella. During the experimental phase, performance parameters, e.g., body weight (BW) and feed intake (FI) were recorded. Differences between control and experimental groups were assessed by using the t-test.

Results: At the beginning of the post-infection period (1, 3, 5 and 7 dpi), all the pigs were Salmonella-positive. There were no significant differences in mean bacterial counts of Salmonella in the feces between the wheat and rye groups. A peak in Salmonella shedding occurred at day 5 after infection in our study. Interestingly, from day 14 onwards after infection, feeding a pelleted diet containing 69% rye showed a significantly lower bacterial count of Salmonella in fecal samples than in those taken from the group fed with 69% wheat in the diet (control and experimental (log10 CFU/g ± SD); 14 dpi: 3.30 ± 0.50 and 2.62 ± 0.18 ($p < 0.001$), 21 dpi: 3.11 ± 0.36 and 2.40 ± 0.65 ($p < 0.001$), 28 dpi: 3.02 ± 0.45 and 2.36 ± 0.57 ($p = 0.001$), respectively). The bacterial counts in the cecal content also differed significantly (control group: 3.34 ± 0.50, experimental: 3.08 ± 0.56; $p = 0.038$). Furthermore, BW and FI did not differ between the groups (control and experimental; final BW (in kg): 27.8 ± 3.72 and 27.7 ± 3.28 ($p = 0.980$), FI (21–28 dpi, in kg): 9.78 ± 1.52 and 9.40 ± 1.48 ($p = 0.136$), respectively. The concentration of total non-starch polysaccharides (NSPs) was 123 g/kg dry matter (DM) in wheat and 140 g/kg DM in rye diets. In addition, the mean concentration of arabinoxylans was 63 g/kg DM and 74 g/kg DM in wheat and rye diets, respectively.

Conclusion: The results of this study support the view that the high proportion of rye might contribute to reducing Salmonella shedding via feces in young pigs from day 14 afterwards. In addition, neither negative nor positive effects of the high ratio of rye diets on performance parameters, e.g., weight gain and feed conversion ratio in young pigs. It can be suggested that up to 70% rye in compound pelleted feed poses no problem for animal health.

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47.

Effects of dietary rye and rapeseed on colonic weight, pH and short chain fatty acids in jejunal and colonic digesta of weaner piglets

Einfluss der Futtermittel Roggen und Raps auf Colon-Masse, pH und kurzkettige Fettsäuren im Chymus des Jejunums und Colons von Absetzferkeln

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Rye and rapeseed meal are alternatives to wheat and soybean meal in pig nutrition. Rye is able to grow under dry climate conditions (1) and has a unique dietary fibre composition (2). Rapeseed is a protein source that is grown in Europe and has positive effects on soil quality. Studies demonstrated that the feeding of higher levels of rye and rapeseed up to 69% and 28% respectively did not impair performance of fattening pigs, but tendentially altered digesta characteristics and bacterial metabolites (3). The aim of this study was to investigate the effect of dietary rye and rapeseed instead of wheat and soybean on colonic weight as well as on pH and short chain fatty acids (SCFA) in jejunal and colonic digesta of weaner piglets. **Methods:** After weaning, a total of 88 piglets were allocated to 44 pens (n=11/treatment). Piglets received four different pelleted diets ad libitum over five weeks. Diets were composed isonitrogenous (ME 14.7-15.8 MJ/kg) and were based on either wheat and soybean meal (T1), wheat and rapeseed meal (T2), rye and soybean meal (T3) or rye and rapeseed meal (T4). Dietary inclusion levels were 48% for rye and wheat, 30% for rapeseed meal and 25% for soybean meal respectively. At the end of the trial, one piglet per pen was euthanised. The relative weight of the emptied colon (% bodyweight) was assessed. Digesta samples were collected from the jejunum and colon descendens. The pH was measured in the digesta and SCFA were analysed via gas chromatography (Agilent Technologies 6890N). The normally distributed data of intestinal pH and colonic weight were analysed using a 2-factorial ANOVA with the factors protein meal and carbohydrate source as well as their interaction. Due to non-normality, data on SCFAs were evaluated by Kruskall-Wallis-test followed by Mann-Whitney-U-test. **Results:** Therelative weight of the colon was higher and the pH in jejunal digesta was lower in rye fed piglets compared to wheat fed piglets ($p < 0.001$; $p = 0.050$). The absolute concentration of SCFA ($p = 0.001$), acetic acid ($p = 0.001$), propionic acid ($p = 0.006$) and n-butyric acid ($p = 0.039$) in jejunal digesta was highest in piglets that received T3. The feeding of T2 resulted in the lowest concentrations of total SCFA, acetic acid and n-butyric acid. Propionic acid was the lowest in piglets of group T1. In colonic digesta, the total concentration of SCFA did not differ between groups, but the feeding of T2 resulted in the highest concentration of n-valeric acid ($p = 0.012$) and T1 and T3 to the lowest concentration. **Conclusions:** The results of this study show that the inclusion of rye increased the organ weight of the colon. In piglets that received a diet based on rye, the reduction of pH in jejunal digesta might be related to the increased amount of SCFA. In the colon, the different diets also altered the concentration of n-valeric acid, but in a less distinct manner than in the jejunum. Further research is needed in order to understand the mode of action of dietary rye and rapeseed on digestive tract development in combination with changes in gut microbiota.

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48.

Effects of winter-faba beans with low vicine/convicine concentrations in the diet on the performance and egg quality of laying hens

Einfluss von Winter-Ackerbohnen mit einer niedrigen Vicin/Convicin-Konzentration im Futter auf die Entwicklung der Leistungen von Hennen und der Eiqualität

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In Germany, faba beans are cultivated as high protein seeds on 56,500 ha and sunflower on 19,000 ha. This is a marginal cultivation in comparison to that of rape seed which has to be justified by its vulnerability towards water shortage during cultivation and because of anti-nutritive seed substances, like tannine, vicine and convicine. In 2015, the BLE initiated the project "Breeding and agronomy of new, vicine poor field beans and use as a local protein feed" to minimize these disadvantages and to enhance the use of the field beans as a feedstuff. The aim of this project was to cultivate a winter field bean with a higher yield potential because of less dry stress and its use in the feed, also of sensitive animal species, like poultry. The following study was carried out to verify the influence of increasing proportions of faba beans with low vicine/convicine (V/C) - concentrations plus high protein sunflower meal (HP 46) as the 2nd protein source in the diet on the performance of laying hens.

Methods: 400 hens (Lohmann Brown) were randomly divided into 4 groups. The hens were kept in pens (20 hens per pen) with 5 pens per group. The study commenced when the hens were 21 weeks old and continued until the end of the 6th laying month (168 days). In the treatments winter field beans concentration (15/30/37%) was increased and sunflower meal (HP 46) decreased (14/9/5%) per kg feed. The influence of the new cultivated poor vicine/convicine (0.045% dry matter) winter field bean was compared with the control group containing only soyabean meal (26%) as protein source in the feed. The vicine/convicine content was reduced by 10-12 times in the new winter field beans compared to rich field beans. Number of eggs laid per pen was recorded daily and the feed consumption monthly. Each month the collected eggs were weighed four times within two weeks. In the 6th laying month, all eggs laid per pen over 3 consecutive days were collected to measure egg composition. Data of laying performance were analyzed with ANOVA (SAS) and the Student-Newman-Keuls-Test ($p<0.05$).

Results: In the 6 laying month the means of laying intensity (89.9 - 91.5%), egg weight (61.3 - 62.6 g/egg) and daily egg mass production (55.8 -56.4 g/hen) was not significantly different between control and treatment groups irrespective of field beans inclusion level. The daily feed intake (119 g/hen) of hens from the 30/37% field beans plus sunflower HP46 9/5% were significantly ($P<0.03$) increased compared to the control (111 g/hen). In the field beans/sunflower groups the feed conversion ratio was 2.06 - 2.13 kg/kg compared to control 1.96 kg/kg. In the 6th laying month the egg composition showed a significantly ($P<0.001$) lower egg weight (63.2 - 64.0 g/egg), a higher percentage of egg yolk (26.6%) and the same egg shell breaking strength (52.4 N) of hens of field bean groups compared with control group (65.7 g egg weight; 25.8% egg yolk; 54.4 N egg shell breaking strength). The body weight of the hens from the 30/37% field beans plus sunflower HP46 9/5% were significantly ($P<0.03$) higher compared to the control hens at the end of the study.

Conclusion: The results revealed that a gradual increase (0/15/30/37%) of vicin/convicin-poor winter field beans in hen feed did not significantly influence laying performance, egg weight and daily egg mass production of hens in the first six laying month compared to the control group (0% field beans).

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49.

Effects of milk replacer allowance on growth performance and oxidative status of dairy heifer calves during an extended preweaning period of 14 weeks

Effekte unterschiedlicher Fütterungsniveaus von Milchaustauscher während einer 14-wöchigen Milchtränke auf das Wachstum und den oxidativen Status von Milchkälbern

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The development of dairy heifers in early life is crucial for their future health and profitability as cows. Conventional rearing of dairy calves is still based on restrictive feeding regimens, i.e. 4 – 6 L/d, or 10% of body weight (BW) and weaning around wk 8 of age to stimulate solid feed intake, promote the development of the rumen, and also to reduce rearing costs. However, increasing the daily allowance of milk replacer (MR) for calves might be better for growth, development, and future milk yields than restricted MR feeding. An improvement of welfare and energy supply can be expected by increasing the allowances for MR and also by extending the time until weaning, because hunger and abnormal behaviors, which are related to restrictive feeding, can be avoided. The objective of this study was to determine the effect of feeding two levels of MR for 14 weeks on growth performance and oxidative stress in dairy heifers. **Methods:** Thirty-seven German Holstein heifers were studied from birth until wk 20 of life. At day 5 p. n., the calves were allocated to a HIGH feeding level (10 L/d, n = 18) or a restricted level (RES; 5.7 L/d, n = 19) of MR (14 % solids; max. 2 L/meal) until gradually weaning at the end of wk 14 of life. The newborn calves were initially kept in individual hutches and were transferred to group pens at 10 d of life. The daily MR and starter intake, as well as the number of rewarded and unrewarded visits, and drinking speed were assessed automatically by the feeding system (Vario Kombi, Förster-Technik GmbH) until weaning. After weaning, the calves were moved to a new group pen and had free access to a total mixed ration for dairy cows. The calves had free access to water and hay throughout the study. Blood samples were taken 36 - 48 h after birth, and subsequently every second week from week 8 to 16, and in wk 20 of life for assessing the concentrations of leptin, adiponectin, and haptoglobin (Hp). The oxidative status was assessed by measuring the antioxidative capacity (FRAP), the oxidative damage of lipids and proteins (TBARS and AOPP), free radicals (indirectly via dROM), and the oxidative stress index (OSi = dROM/FRAP*100). Health checks and BW records were performed weekly. Statistical analyses using SPSS were performed using a linear mixed model considering group, wk of life (time) and the interactions thereof as fixed effects, and calf as random effect. Significance was declared at $P < 0.05$. **Results:** HIGH fed calves had greater pre- and postweaning BW until wk 20 of life. The average daily gains (ADG) increased from wk 3 and decreased postweaning in both groups, whereby the RES calves had a more pronounced growth depression than HIGH in wk 16. The intake of starter did not differ between the two feeding groups and the higher intake of metabolizable energy (ME) in HIGH was maintained until weaning by MR intake. The RES calves visited the feeder more often and had more unrewarded visits than HIGH calves throughout the milk feeding period. Adiponectin, an insulin-sensitizing hormone, was influenced by time and feeding level. Leptin was only influenced by time, but not by feeding group. Health status and Hp concentrations in serum were not different between the feeding groups. Values of dROM, FRAP, and OSi increased with age, whereas TBARS and AOPP decreased. Group effects were limited to AOPP and FRAP, both variables with greater concentrations in RES than in HIGH; this difference in FRAP was already apparent in new-borns. **Conclusion:** The HIGH feeding level improved animal welfare by reducing signs of hunger, while starter intake was not compromised. The differences in FRAP which are already seen in new-borns and were thus rather not related to the feeding level. Greater AOPP concentrations in RES may indicate a greater portion of oxidized proteins in RES, but the relevance of this finding is yet unknown. It remains open to what extent the positive effects of HIGH feeding on BW and ADG would determine the future performance as a cow.

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50.

Influence of slaughter age and dietary energy concentration on the empty body nutrient contents of growing Fleckvieh bulls

Einfluss des Schlachtalters und der Energiekonzentration in der Ration auf die Nährstoffgehalte der Leerkörper wachsender Fleckviehbullen

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Performance of Fleckvieh (German Simmental) fattening bulls has been improved by selective breeding during past decades. This might have affected carcass tissue composition as well as chemical body composition and as a consequence energy and nutrient requirements of animals during fattening might have changed. In order to reevaluate the deposition of energy and nutrients and the chemical and body tissue composition of growing Fleckvieh bulls of modern type, a feeding and slaughter experiment was conducted. This abstract presents data on nutrient content of bulls slaughtered in different weight categories after feeding diets with different energy concentrations. **Methods:** 72 Fleckvieh bulls (age: 42 d, body weight (BW) 80 kg) were fed with restricted amounts of milk replacer (120 g/l) with a maximum of 6 l/d and a total mixed ration (TMR) based on concentrates (55.7 %), hay (30.0 %) and molasses (14.3 %) over a period of 6 weeks until weaning at an average BW of 121 kg. Subsequently, the animals were fed a TMR based on maize silage (average 63.6 %), concentrates (30.8 %), hay (3.7 %) and molasses (1.9 %) for ad libitum intake. The TMR for the period after weaning (8 weeks) was adjusted weekly and supplemented with brewer's yeast, 110 g per calf and day. The fattening period began at an average BW of 225 kg. Bulls were randomly allocated to a normal and a high energy treatment group with 11.6 and 12.4 MJ ME/kg DM, respectively. Individual feed intake was recorded daily and BW was determined in four-week intervals. The bulls were slaughtered in five final live weight groups with 120 (n=8), 200 (n=10), 400 (n=18), 600 (n=18), and 780 kg (n=18). During slaughtering and carcass processing, the empty body weight was determined as final live weight minus the contents of urinary bladder and gastrointestinal (GI) tract and the whole empty body was dissected to the body tissue fractions hide, blood, organs, empty GI tract, body fat, muscle, bone and tendon. Body tissues were chemically analyzed for crude fat, crude protein, crude ash and water contents. Statistical analysis was performed using Proc Mixed of SAS (Version 9.4). Results are shown in ranges and standard error and were compared by the PDIFF option with values of $p<0.05$ regarded as significant. **Results:** The empty body weights of weight groups 120, 200, 400, 600, and 780 kg were 104, 176, 370, 553, and 734 kg, respectively. Since there were no significant effects of dietary energy concentration on nutrient contents in normal and high energy treatment groups, the combined results of both animal groups are shown. During growth, the percentage of crude protein, crude ash and water decreased ($p<0.05$; crude protein: 20.6-19.1 % ±0.2; crude ash: 4.8-4.4 % ±0.1; water: 68.4-55.3 % ±0.5), while the crude fat percentage increased ($p<0.05$) from 6.2 to 21.3 % ±0.7. **Conclusions:** Variations in dietary energy concentrations within margins found under practical conditions did not alter the body nutrient composition to a relevant extent. The body nutrient contents of modern type Fleckvieh bulls corresponded widely to literature data from past decades (1). During growth, the amount of crude fat increased mainly at the expense of body water. Furthermore, modern bulls showed a 600 g higher daily weight gain during the fattening period and thus had a higher nutrient accretion than bulls in former studies (2). In summary, modern type Fleckvieh bulls feature a higher growth potential and can be fattened up to 780 kg final live weight. **Acknowledgement:** This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727213 (GenTORE).

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51.

Effect of diet change and duration on a high grain ration on rumen pH, saliva composition and salivation in cows supplemented or not with a phytogenic feed additive

Einfluss eines phytogenen Futtermittelzusatzes auf den Pansen pH-Wert, die Speichelproduktion und -zusammensetzung bei trockenstehenden Milchkühen während einer erhöhten Kraftfuttergabe

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High grain diets contribute to enhance milk yield in dairy cows. However, these diets may negatively affect ruminal pH and salivation, and the effects on saliva composition are not elucidated yet. Additionally, phytogenic compounds have been suggested to enhance salivation; however, there is limited information on their impacts in dairy cattle. Therefore, this research evaluated the effect of the change from a forage to a high grain diet, and the duration of consumption of the high grain ration on ruminal pH, saliva composition and salivation in cows supplemented with or without a phytogenic feed additive (PFA). **Methods:** Nine non-lactating cannulated Holstein dairy cows were used in a change-over design. Cows were divided in two blocks of 4 and 5 cows and were supplemented with either a control neutral carrier or with a PFA consisting of a mixture of menthol and thymol. During each experimental run, cows were fed a forage diet for 1 week (45% grass silage, 45% corn silage and 10% grass hay), then they were transitioned to a high grain diet (26.25% grass silage, 8.75% corn silage and 65% concentrate), which they consumed for 4 weeks. Between the 2 experimental runs, there was a washout period of 10 weeks, where cows grazed on pasture to fully recover. Rumen pH was measured continuously every 15 min with indwelling systems. Saliva samples were collected orally every week by suction using a vacuum pump for analyses of bicarbonate, phosphate, mucin and lysozyme activity. Salivation was measured on the week of forage feeding and on week 4 of grain feeding by collection of feed boli from the cardia over a 30-min interval. Data were analysed with the Proc Mixed procedure of SAS with week of feeding and treatment as fixed effect and cow as random effect. Treatment means were compared using the PDIFF option. **Results:** Ruminal pH decreased ($P < 0.05$) with the change to high grain diet. Within the grain feeding period, ruminal pH reached its lowest value during week 1 (6.45, 6.06, 6.16, 6.16 and 6.15 ± 0.052 for the week of forage feeding and the 4 weeks of grain feeding, respectively). During week 3 of grain feeding, there was a tendency ($P = 0.08$) for PFA to increase rumen pH (6.10 and 6.23 ± 0.051 for control and treatment, respectively). On the other hand, saliva bicarbonate was not affected by diet change ($P = 0.91$). However, within the grain feeding period ($P < 0.05$), the lowest level was observed on week 4 (77.3, 77.0, 82.3, 76.7 and 74.2 ± 1.82 mM, respectively). Saliva phosphate increased ($P < 0.05$) with diet change; within the grain feeding, the greatest value was observed on week 4 (10.7, 11.5, 11.3, 11.7 and 12.9 ± 0.84 mM, respectively). Salivary mucin ($P = 0.61$) was not affected by diet change or duration of grain feeding and averaged 1.04 mg/mL. No effect of PFA ($P \geq 0.18$) was found on bicarbonate, phosphate or mucin. Lysozyme activity was not affected by diet change ($P = 0.14$); within the grain feeding period, the maximum value ($P < 0.05$) was observed on week 3 (39.7, 44.8, 41.2, 60.4 and 50.5 ± 8.62 U/mL/min, respectively). In week 3 of grain feeding, lysozyme activity was greater ($P < 0.05$) for PFA compared to control (86.70 and 34.18 ± 8.62 U/mL/min, respectively). Furthermore, the change to grain diet decreased ($P < 0.05$) feed ensalivation (5.2 and 2.9 ± 0.33 g saliva/g feed DM), with no effect of PFA ($P = 0.61$). However, saliva flow rate was not affected by diet change ($P = 0.31$) or PFA ($P = 0.40$) and averaged 74.2 g/min. **Conclusion:** Ruminal pH and feed ensalivation decreased with the change to high grain diet. The duration of consumption of a high grain diet influenced salivary bicarbonate, phosphate and lysozyme activity. Results also suggest that on the third week of high grain feeding, the PFA enhanced ruminal pH and the activity of lysozyme, a salivary component known to have antimicrobial properties.

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52.

Investigations on feeding and rumination behavior in goats and sheep

Untersuchungen zum Fress- und Wiederkauverhalten von Ziegen und Schafen

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It is often assumed that the feed intake behavior and rumination patterns of common domestic ruminants (sheep, cattle and goats) are identical across species. Comparative studies have mostly been conducted in sheep and cattle. In a review comparing sheep and goats, it was reported that goats have more feeding bouts and feed longer per unit of dry matter intake (DMI) than sheep, while they spent less time ruminating compared to sheep (1). Therefore, it seems that the feeding and rumination behavior of goats is somewhat different compared to sheep. Consequently, this study was designed to investigate differences in feeding and rumination behavior between goats and sheep in a systematic fashion. **Methods:** In a preliminary experiment, four goats and four sheep were offered grass hay (61 g XP, 696 g aNDForg, 406 g ADForg; all per kg DM) for ad libitum intake. Sheep and goats were housed in two separate pens, fitted with Calan gates that allowed individual feed intake measurements. The DMI was measured over five consecutive days. The feeding and rumination behavior was recorded using video cameras. In the main experiment, sheep and goats were offered grass hay cut to two different chop lengths in a 2 × 2 factorial design. The experiment was divided into ten days of adaption and five days of data collection. Like in the preliminary experiment, goats and sheep were housed in separate pens equipped with Calan gates and cameras, and were fed for ad libitum intake. The trial hay (101 g XP, 692 g aNDForg, 381 g ADForg; all per kg DM) was chopped to two mean particle lengths: "long" (~18mm) and "short" (~7mm). Individual DMI was determined over five consecutive days; the feeding and rumination behavior was evaluated for two days from the video recordings. Results of the preliminary experiment were analyzed descriptively. Data of the main experiment were analyzed using the mixed procedure of SAS (Version 9.4) (2 observations/animal). The model included the fixed effects of species, chop length and their interaction. Animal was considered as random effect. Treatment means were compared by Tukey-Kramer test. Significance level was set at $P<0.05$. **Results:** In the preliminary experiment, sheep spent 211±51 min/d eating, while goats needed 250±41 min/d for feed intake. Goats ruminated for 529±49 and sheep 581±80 min/d. In the main study, goats spent more time eating (275 min/d) the short hay compared to sheep (175 min/d; $P<0.05$) but there were no differences in the duration of total daily rumination time. When fed the longer hay sheep spent more time eating (222 min/d) compared to the short hay (175 min/d; $P<0.05$). Goats consumed more long than short hay (1827 vs. 1440 g DM/d, $P<0.001$). The chop length of the hay had no impact on the DMI of the sheep (short hay: 1558 g/d, long hay: 1544 g/d). Per kg LM0.75 sheep consumed 57 g DM short hay respectively 56 g DM (± 9.9 g) long hay while goats ate 55 g/kg LM0.75 short hay and 70.6 g/kg LM0.75 long hay ($P<0.001$). In sheep, the proportion of rumination to eating was 3.4:1 for the short hay and 2.7:1 for the long hay ($P<0.001$). The chop length of the hay had no effect on the proportion of rumination to eating time in goats. **Conclusion:** In this study, goats had a higher DMI of long hay than of short hay while sheep showed no significant differences. Sheep spent more time eating the long hay than the short hay. In contrast to (1), goats did not seem to have a shorter rumination time than sheep; however, a difference in the ratio of rumination:feeding was present.

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53.

Effect of a commercial tannin feed additive on the nitrogen balance of goats fed a low-protein diet

Wirkung eines kommerziellen Tannin-Futteradditivums auf die Stickstoffbilanz von Ziegen bei proteinarmer Fütterung

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Including tannins in ruminant diets can improve live weight (LW) gain, milk and wool production and reduce gastro-intestinal parasites (Waghorn, 2008). Moreover, protein use efficiency may be enhanced and nitrogen (N) excretion be shifted from urine to faeces, potentially reducing gaseous N emissions from animal excreta (Hess et al., 2006). Yet, effects depend on the type of tannin(s) and of dietary protein.

Methods: A feeding trial was conducted on the experimental farm of Sultan Qaboos University in Muscat, Oman. In a 4 (treatments) x 3 (periods) x 2 (animals) Youden square, two weaned Batinah bucks each were fed a high or low roughage diet consisting of Rhodes grass hay and crushed barley at 70:30 and 50:50 ratio (on dry matter (DM) basis), with or without addition of a commercial Quebracho tannin feed additive (2 g/kg0.75 LW; Silvafeed® ByPro, SilvaTeam, San Michele Mondovì, Italy). Rations used reflect common goat feeding practice in Oman. Each period comprised 11 d adaptation and 7 d sampling. Total feed refusals, feces and urine were collected daily and samples pooled per animal and period for laboratory analysis. A mixed model ANOVA with animal as random effect and period, roughage level, tannin addition and their interaction as fixed factors was computed after testing homogeneity of variances and normal distribution of residuals (SPSS V27.0.0.0, IBM Cooperation).

Results: Due to their young age and low LW, feed intake of goats was relatively low. The concentration of crude protein in dry Rhodes grass hay and barley was 7 and 10 g/100 g DM, which together with the low feed intake resulted in a low N intake. Roughage level (not shown) and tannin addition (Table 1) had no effect on feed DM and N intake, DM digestibility, fecal and urinary N excretion (p = 0.22-0.89).

Table 1 Live weight (LW), daily dry matter (DM) and nitrogen (N) intake, DM digestibility (DMD), daily fecal and urinary N excretion and daily N balance of goats fed Rhodes grass hay and barley without (n=12) or with (n=12) a commercial Quebracho tannin feed additive.

Tannin	LW	Tannin additive	DM intake	N intake	DMD	N Feces	N Urine	N balance
feed	(kg)	(kg/kg DM)	(g/kg	(g/kg)	(g/kg			
No	14.4	0	38.0	0.55	620	0.30	0.12	0.13
Yes	13.6	62	36.2	0.50	655	0.28	0.14	0.08
SEM	0.59	7.3*	2.11	0.03	23.1	0.02	0.01	0.03
p-value			0.69	0.45	0.22	0.27	0.41	0.89

Values depict arithmetic means and standard error of the mean (SEM); *SEM for tannin treatment only. There were no effects of tannin supplementation, roughage level, and their interactions. # Homogeneity of variances was not met. + Normal distribution of residuals was not met.

Conclusions: Irrespective of diet composition, no beneficial effects of the tannin additive could be confirmed with the N-poor rations tested in the present study. Under such conditions, only potential health benefits of the additive, such as reduction of parasite burden, might be of interest to goat keepers.

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54.

Immunoglobulin G and colostrum composition in dairy and dual-purpose cattle

Immunglobulin G und Kolostrumzusammensetzung bei Milch- und Zweinutzungskühen

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Colostrum contains high concentrations of immunoglobulin (Ig) G, protein, fat and other bioactive compounds compared to mature milk. Colostrum of dairy cows is assumed to be of poorer quality compared to beef cows. The objective of the present study was to compare colostrum composition of different cattle breeds that are considered dairy and dual-purpose types. We further evaluated the impact of parity number, milk yield of the previous lactation, gestation and dry period length, and time of first milking relative to parturition on the concentration of selected colostrum components.
Methods: Colostrum samples of 458 cows from 13 different breeds were collected by farmers in Switzerland and Germany. At least 5 colostrum samples of one breed were provided by one farm. In total, 28 dairy farms contributed to this field study, with one farm per breed (Rhetic Gray, 5 cows) up to 10 farms (German Fleckvieh, 177 cows). Colostrum samples (approximately 50 mL) were obtained from the first milking after parturition (4.1 ± 3.7 h after parturition; range from 0 to 15 h) and immediately frozen at -20°C until analysis. In addition, participating farmers provided data about the individual calving cows (e.g., parity number, date of insemination and date of dry-off, previous lactation yield), time of parturition and first milking. Total IgG concentration in colostrum was analyzed with a modified ELISA kit (no. E10-118; Bethyl Laboratories Inc.). Colostrum samples were diluted in ELISA wash buffer (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, adjusted to pH 8.0) to final dilutions of 1:800,000 and 1:1,600,000. The analysis of fat, protein and lactose concentrations was carried out by a milk infrared analyzer (MilkoScan 7 RM, Foss Analytical A/S, Hillerød, Denmark). For the evaluation of associations of various parameters on IgG, fat, protein, and lactose concentrations in colostrum, a generalized linear model (GLM) procedure with breed and parity number as class variables and additionally either gestation and dry period length, or previous lactation yield as individual covariate was used. Significant effects of breed and other variables, respectively, were detected by the Tukey-Kramer post-hoc test at $P<0.05$. The impact of the interval length between parturition and time of first milking on colostral IgG concentration was evaluated with a GLM procedure with time relative to parturition as fixed effect.
Results: The IgG concentration varied broadly between and also within breeds, ranging from 12.7 mg/mL to 204.1 mg/mL. We observed the highest average IgG concentrations in two dual-purpose breeds, Montbéliarde (123.6 ± 43.6 mg/mL) and Original Braunvieh (116.4 ± 28.6 mg/mL, followed by two high-yielding dairy breeds, German Holstein Friesian (110.5 ± 39.0 mg/mL) and Brown Swiss (110.2 ± 34.0 mg/mL). Lowest IgG were measured in Murnau-Werdenfels dual-purpose cows (51.0 ± 20.3 mg/mL). Previous lactation yield was not related to IgG concentration in colostrum ($P>0.05$). We observed highest IgG concentrations in colostrum milked within 3 h p.p. Up to approximately 9 – 12 h p.p. colostral IgG content remained rather constant. After approximately 12 h, IgG concentration in colostrum declined concomitantly with the start of copious milk production, reflected by a significant rise in lactose concentration. Similar to IgG, also the contents of fat, protein and lactose in colostrum showed wide ranges within and among breeds, from 1.10 to 20.88%, 3.34% to 26.50%, and 1.61% to 4.60%, respectively. On average over all breeds, we observed results of 5.93 ± 3.09% for fat, 14.04 ± 3.70% for protein, and 2.95 ± 0.56% for lactose. IgG and protein concentrations were higher in multiparous compared to primiparous cows ($P<0.0001$). In contrast, fat and lactose contents were greater in first lactating cows ($P<0.0001$).
Conclusion: Due to limitations of this study, the effect of the individual farm on colostrum characteristics within breed cannot be excluded. The present study confirms the significant variation in IgG and other colostrum constituents within and among breeds. Although differences between cattle breeds were detected, high-yielding dairy cows did not have poorer colostrum quality compared to lower yielding animals such as beef and dual-purpose breeds.

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55.

Effects of grazing intensity of beef heifers on growth performance and pre-slaughter physiological stress reactions and their consequences for meat quality

Effekte der Weide-Intensität von Mastfärsen auf Leistung und physiologische Reaktionen vor der Schlachtung und ihre Konsequenzen auf die Fleischqualität

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Grazing management has shown to affect growth performance (1) which may potentially have consequences on carcass and meat characteristics. Behavioural and physiological stress reactions during the pre-slaughter period may also influence meat quality (2). The present study investigated the effects of grazing intensity including stocking density and rotation frequency on growth performance and meat quality of beef heifers while taking into account their pre-slaughter physiological status.

Methods: Thirty-two disbudded corssbred heifers (Limousin × dairy breed), initially weighing 421 ± 30 kg and aged 12.5 mo, were reared from May 2017 to September 2017 in a rotational grazing system on pastures at 1200 m above sea level. The animals were kept in four groups of eight heifers each on a total surface of 22 ha. Two of the four groups rotated on a total surface of 11 ha, divided into eight paddocks with a surface of 72 a each (low intensity, LI), while the other two groups rotated on the other 11 ha, divided into 24 paddocks with a surface of 24 a each (high intensity, HI). stocking density and number of paddock rotation the HI groups changed paddocks 3 times more often than LI groups to respect the target postgrazing sward height of 5 cm. Thus, grazing intensity referred to differences in paddock size, i.e. stocking density, and number of rotation between paddocks, but the total grass allowance was the same for the four grazing groups. During the grazing period, animals were weighed monthly. The heifers were slaughtered in September 2017. Pre-slaughter physiological status was assessed by measuring heart rate (HR; from loading on farm to stunning) and salivary cortisol (before loading and at stunning). Carcass weight and CH-TAX (equivalent to EUROP) classification (conformation and fat cover) (3) were assessed within 30 min after slaughter. Meat quality measurements included early pH and temperature decline (1-6 h post mortem (pm)), ultimate pH (48h pm), water losses (at maturation, thawing, cooking) and shear force 48 h and 14 d after maturation. For statistical analysis, linear mixed models and correlation analysis were used.

Results: Grazing intensity had no effect on daily weight gain (mean overall daily weight gain: 0.18 ± 0.12 kg/d), live weight at slaughter (mean: 446 ± 30 kg), carcass weight (mean: 240 ± 17 kg), fat cover and carcass conformation ($P>0.10$). During the pre-slaughter period, LI heifers tended to higher salivary cortisol concentrations before loading ($P<0.10$), had lower HR at loading (-14%, $P<0.05$), and had higher HR during the last ten minutes in the truck, and from unloading to stunning (+14 and +19%, respectively, both $P<0.05$). Early pH (1 h and 4 h pm) was lower (both $P<0.05$) in LI than in HI heifers (1 h pm: -1%; 4 h pm: -2%). Cooking loss after maturation was higher ($P<0.05$) in LI heifers than HI heifers (+8%). No differences between groups were found in early pm temperature and shear force ($P>0.05$). For both LI and HI groups, heart rates 10 - 0 min before slaughter were negatively correlated ($r=-0.51$, $P<0.05$) with early pm pH. For LI heifers, pH decline was positively correlated ($r=0.72$, $P<0.05$) with cooking loss after maturation.

Conclusions: Grazing intensity did not affect growth performance and carcass characteristics in heifers. Compared to heifers grazing at a higher grazing intensity, those grazing at a lower intensity showed greater stress levels during the pre-slaughter period, which contributed to lower early pH and greater cooking losses of the meat.

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56.

Impact of elevated dietary levels of grass silage and its supplementation with maize silage or corn-cob mix on performance, carcass and meat quality of growing bulls

Einfluss erhöhter Anteile an Grassilage in Kombination mit Maissilage oder Corn-Cob-Mix im Futter auf Mastleistung, Schlachtkörper- und Fleischqualität von Mastbullen

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Intensive beef production in Switzerland is mainly based on maize silage and concentrate but does not meet the standards of the recently implemented Swiss national programme promoting grassland-based milk and meat production. Due to the lower energy density of grassland-derived feeds, an impaired animal and slaughter performance is anticipated when extending the use of grass silage in intensive beef fattening. The aim of this study was to evaluate the effects on performance, carcass and meat quality of growing bulls fed diets with increasing proportions of grass silage combined with either maize silage or dried corn-cob mix (CCM) and a reduced amount of concentrate compared to a common diet type.
Methods: Thirty Limousin-sired bulls (initial body weight (BW): 164±3.5 kg (mean±SEM)) were randomly assigned to one of five diets composed of grass silage, maize silage and concentrate in ratios of 0.1:0.6:0.3 (G10; control), 0.3:0.5:0.2 (G30) and 0.5:0.3:0.2 (G50), or grass silage, CCM and concentrate at 0.5:0.3:0.2 (G50CCM) and 0.75:0.15:0.1 (G75CCM), respectively. The control group was fed a commercial beef cattle concentrate (27% crude protein (CP)), whereas all other groups were fed a grain-based concentrate (14% CP). Every two weeks, the animals' BW and individual feed intake were recorded. Bulls were slaughtered at 521±2.0 kg BW. Carcass conformation and fat cover were evaluated according to CH-TAX (equivalent to EUROP). Samples of the Longissimus thoracis were aged for 21 days before assessing the meat quality including water holding capacity, Warner-Bratzler shear force and lightness, redness and yellowness. Data was statistically analysed in RStudio (version 1.2.5001) performing an ANOVA or Kruskal-Wallis test for nonparametric data (cooking loss and yellowness).
Results: Days on experimental diets differed ($P < 0.001$) according to different ($P < 0.001$) average daily gains, which were higher in bulls fed G10 (247 days; 1.43 kg) and G50CCM (270 days; 1.34 kg) than in bulls fed G30 (314 days; 1.15 kg), G50 (300 days; 1.20 kg) and G75CCM (305 days; 1.17 kg). According to the experimental design, average forage dry matter intake (DMI) was lower ($P < 0.01$) in G10 than in G50CCM and G75CCM. As intended, average concentrate DMI was highest ($P < 0.001$) in G10 and lowest in G75CCM. However, average total DMI (6.15±0.099 kg) was similar among diets. A more favourable ($P < 0.001$) feed conversion ratio was found in G10 (not different from G50CCM), whereas G30 required most feed for gain. The CP intake was lowest for G30 ($P < 0.001$) and highest for G10, but G50CCM and G75CCM were not different from G10. Net energy intake was highest for G10 and G50CCM ($P < 0.001$), whereas it was lowest for G75CCM. Bulls of G10 and G50CCM had a higher ($P < 0.001$) intake of metabolisable protein than the other groups. Carcass weight, dressing percentage, conformation and fat cover score remained unaffected by the diet. Regarding absolute values, all groups achieved the ideal fat cover score of 3, except for the control group (score 2). Ageing, drip and cooking loss of the meat were comparable among diets. Meat lightness was similar, whereas yellowness tended to vary ($P < 0.1$) between groups. Meat of bulls fed G50CCM was redder ($P < 0.05$) than that of bulls fed G10. Shear force was lower in G75CCM ($P < 0.05$) than in G10.
Conclusion: These findings show that the amount of grass silage can be increased up to 50% in the diet when complemented with CCM, while limiting the amount of concentrate without any adverse effects on animal performance, carcass and meat quality. The comparably better performance of G50CCM to G50 can be explained by the higher energy density in CCM. Thus, this diet type could be a suitable alternative to common maize silage and concentrate-based fattening diets.
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57.

Observational field study with sheep on pastures with common ragwort (*Senecio jacobaea L.*) from an animal health and nature conservation perspective

Beobachtungs-Feldstudie zur Schafbeweidung von Grünland mit Jakobs-Greiskraut (Senecio jacobaea L.) aus tiergesundheitlicher und naturschutzfachlicher Sicht

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Species-rich pastures naturally contain potentially toxic plants. Therefore, those pastures are classified as a risk for animal health. One environmentally friendly option to reduce common ragwort (*Senecio jacobaea L.*) is grazing by sheep. In contrast to other livestock, sheep not only readily eat fresh ragwort, but tolerate its toxins (pyrrolizidine alkaloids [PA]) better than other livestock species. No evidence-based risk assessment has been carried out, nor are data available on the chronic impact of PA in sheep eating ragwort on ordinary pastures. Here, we document (1) to what extent sheep ingest ragwort compared to other plant species and (2) what grazing behaviour under free-choice conditions means for the individual animal's health.

Methods: In May 2020, 70 sheep were purchased. Seven animals were slaughtered and investigated as a control group, and the rest was placed on a 5.25 ha extensively cultivated pasture at Höltigbaum, Hamburg. From May until October four groups of seven sheep each were slaughtered at intervals of 6 weeks, and the size of the pasture was reduced accordingly to maintain a stocking density of 12 sheep/ha. 35 individuals remained at the end of October 2020. At slaughter, blood and liver samples were taken to measure haematologic parameters, enzyme activity and liver copper content. Liver tissue samples were used for histopathological evaluation. During grazing health of the sheep was continuously monitored, including faecal egg count. 81 plots of 4.47 m × 4.47 m were established to observe grazing behaviour two times monthly. All missing (= ingested) parts of ragwort as leaves of rosettes and stems were counted. An estimation of the biomass as well as a Weende analysis of both vegetation and ragwort plants were carried out.

Results: Sheep consumed all parts of ragwort throughout the whole vegetation period. Overall intake increased from June until August. Starting with rosette leaves in May (ragwort biomass=30%), they switched to shoots and leaves and even stalks from June on (biomass 54%). In the beginning of September ragwort made up only 14% of the plant biomass, but regrowing rosette leaves increased this share to 32% until the end of the month. Surprisingly, old stems damaged by grazing produced new flowering shoots while sheep were still grazing on the plot.

Within the first four weeks of pasturing each animal consumed about 2750 half and 2500 whole rosette leaves on average, which corresponds to 907.5 g and 1850 g of ragwort, respectively.

So far, no significant impact on animal health has been observed. Behaviour and body condition scores remained unchanged, irrespective of the time of exposure to common ragwort. Signs of photodermatitis could not be observed. Haematology and blood biochemistry parameters remained within the reference limits, with the exception of serum γGT activity and liver copper content, which were already elevated before the animals went on pasture. All animals including the control group had slight to moderate hepatitis, fibrosis and proliferation of the bile ducts but no morphological signs in respect to cirrhosis of the liver.

Conclusion: Sheep are seemingly capable of ingesting large amounts of common ragwort within one grazing period without showing any significant medical issues. From a nature conservation point of view, it is not yet clear whether sheep are an option to control common ragwort efficiently. Thus, we urgently need data from the second grazing period in 2021.

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58.

Investigations on the transfer of non-dioxin like polychlorinated biphenyls from feed into hen eggs

Untersuchungen zum Transfer von nicht-dioxin-ähnlichen Polychlorierten Biphenylen aus dem Futter in die Eier von Legehennen

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In a recent event, non-dioxin-like polychlorinated biphenyls (ndl-PCBs) were detected in individual samples of feed, eggs, turkey and chicken meat in concentrations above the maximum levels (1). Consequently, the need arises to quantitatively relate the concentrations in specific foods of animal origin to the concentrations in feed. In particular, limited data are available on the transfer of individual ndl-PCB congeners from animal feed into hen eggs. Taking into consideration recent and past contamination events, it is necessary to carry out targeted transfer studies with laying hens and defined ndl-PCB contents and congener patterns in animal feed.

Methods: A total of 30 laying hens were divided into two groups (A and B) of 15 animals each and kept in groups under floor housing conditions. After an adaptation period of 21 days, both groups received a commercial compound feed contaminated with ndl-PCBs (11.50 ± 1.27 µg/kg at 88% DM) from paint of loading cells of a feed company. The hens of group A received the feed for 28 days and the hens of group B for 62 days. Afterwards, both groups underwent a 100-day depuration period with a conventional compound feed with a very low ndl-PCB background contamination (control diet, 0.19 ± 0.02 µg/kg ndl-PCB at 88% DM). The eggs of both groups were collected and weighed daily. The yolks of each group were pooled for pre-selected days allowing subsequent development of a physiologically based pharmacokinetic (PBTK) model for ndl-PCB in the laying hen. Analysis of ndl-PCB in the samples were performed by gas chromatography and high-resolution mass spectrometry.

Results: The ndl-PCB levels in eggs exceed the currently maximum permitted level of 40 ng/g fat ndl-PCB already after 10 days of feeding the contaminated feed (1). In addition, ndl-PCB levels in egg exceeded regulatory limits by 2.6-fold after 62 days of feeding the contaminated diet in group B. Feeding the control diet resulted in declining ndl-PCB concentrations in eggs. Thus, the levels of ndl-PCBs dropped below the maximum level (1) after 9 and 20 days for groups A and B, respectively. However, a relatively long depuration period was observed including differences among individual ndl-PCB congeners, where the congeners PCB28, PCB138, PCB153, and PCB180 showed slower rates as compared to PCB52 and PCB101, respectively. To elucidate the congener-specific transfer properties of ndl-PCBs, physiologically based toxicokinetic (PBTK) models were derived. The properties given by these models include the alpha- and beta- egg elimination half-lives, as well as the feed-to-egg transfer factor and transfer rate for each studied congener. To evaluate the minimum depuration period required for a contamination event that differs from the current experimental design, a model simulation would be required because the result is dependent on congener pattern, duration of exposure and background contamination levels.

Conclusions: The present study re-establishes a considerable transfer of ndl-PCB from feed to food of animal origin. The developed PBTK model revealed a congener-specific pattern. The model may thus help to understand congener-specific transfer and depuration scenarios in feed contamination events as a decision-making tool.

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59.

Investigations on undesirable substances in, and the possible oral uptake of interspersed substrates by pigs

Untersuchungen zu unerwünschten Stoffen in, und die mögliche orale Aufnahme von Einstreumaterialien durch Schweine

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In Europe, minimum standards exist for pig husbandry regarding the availability of appropriate materials enabling exploration activities (1). Indeed, a large variety of materials is available to enrich the animals' environment and to prevent behavioral disorders. Many of these enrichment materials meet further requirements for animal welfare such as providing comfortable bedding and a clean and dry environment. The transition between fields of application of these interspersed substrates is often fluent. Interestingly, main characteristics for these substrates include "chewable" and "edible" (2). Hence, pigs may consume a part of the provided material, which consequently contributes to the pigs' daily ration. Here, we analyzed a set of 71 different interspersed materials for their content of undesirable substances and performed an observational study to investigate the preference of pigs for certain materials.

Methods: A total of 71 materials (disinfectant powders [n=51], earth/peat [n=12], biochar [n=8]) used as bedding, enrichment or hygienic material in pig husbandry were analyzed for their content of heavy metals (As, Pb, Cd, Hg), trace elements (Fe, Cu, Zn, Ni, Cr), dioxins and polychlorinated biphenyls using standard analytical procedures (DIN EN ISO 17294-2:2017 and 15763:2010-04, DIN EN 16215).

To study the possible consumption of the material, a camera-assisted observational experiment was conducted. A total of twelve female pigs (German Landrace) in groups of two animals per pen (six pens) were used in a 4 x 6 factorial arrangement to investigate their preference for a disinfectant powder, peat, biochar and straw as reference material. The materials were presented to the pigs as a combination of two materials per pen over a five-day period. After a material-free two-day period, a new combination was offered to the animals. Within six weeks, all groups had received every material combination (Latin square design). Video recordings from day one and day five of each period, respectively for all pigs were analyzed focusing on frequency and total time of interaction with the different materials. Additionally, in order to examine whether the pigs might ingest offered materials, feed, peat (most preferred material by pigs in the literature [3]), straw and fecal samples from pigs receiving the peat-straw combination were analyzed for long-chain n-alkanes (in a range of 25 to 36 carbon atoms) naturally occurring in the provided litter using gas chromatography.

Results: Some materials (especially disinfectant powders and earth/peat) contained considerably high levels of heavy metals (up to 22, 828 and 11 mg/kg for As, Pb and Cd, respectively), trace elements (up to 84, 5 and 3 g/kg for Fe, Cu and Zn, respectively) and dioxins (up to 3.8 ng/kg WHO-TEQ for PCDD/F-PCB). Considering an oral uptake of interspersed materials as part of the daily ration, certain substances in these materials might exceed maximum permitted levels for feed. In this context, the observational study revealed that the animals explored and partly consumed all tested materials. The highest preference was determined for earth/peat, followed by biochar, straw and disinfectant powder. Furthermore, long-chain n-alkanes from earth/peat and straw were detected in pig manure, whereas the feed contained hardly any of these n-alkanes. Thus, the observational study and fecal analyses underpin the possible oral uptake of interspersed materials by pigs.

Conclusions: Due to a potential oral uptake of interspersed materials by pigs, undesirable substances in these materials may enter the food chain or affect animal health. A quantitative risk assessment is yet not possible. Further studies will address the quantitative contribution of undesirable substances by oral intake of interspersed materials to the daily ration.

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60.

Feeding waste milk as a risk factor for the occurrence of fecal extended spectrum beta-lactamase (ESBL)-E. coli in pre-weaned calves on large dairies in Germany

Fütterung von Sperrmilch als Risikofaktor für das Vorkommen von fäkalen Extended Spectrum Beta-Laktamase (ESBL)-E. coli bei Saugkälbern auf großen Milchviehbetrieben in Deutschland

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Usually fecal Escherichia (E.) coli of cattle carry less antimicrobial resistance (AMR) than fecal E. coli of other farm animals. However, young calves have higher prevalence of extended spectrum beta-lactamase (ESBL)-E. coli than young stock and adult cattle (1). Worldwide, ESBL-E.coli are one of the most important multi-resistant bacteria (2). AMR of commensal microorganisms is mostly related to antibiotic treatment. In calves, feeding waste milk containing antimicrobial residues could be a risk factor for higher prevalence of ESBL-E.coli. However, the results of several studies are varying. For Germany, there is only one study dealing with ESBL-E.coli prevalence in small dairy and beef herds in Bavaria, which could not detect effects of feeding waste milk but ESBL-prevalence significantly correlated with herd size (3). Therefore, the objective of the present study was to detect risk factors for the prevalence of fecal ESBL-E.coli in young pre-weaned calves on large dairies in Germany.

Methods: Overall, 72 dairy farms with average 667 milking cows, respectively, were included in the study. Representative fecal samples were taken of calves between 7 and 28 days of life. Overall, we examined samples of 1442 calves. Detection of ESBL-E.coli was done by Cefotaxim-containing CHROMagar. Furthermore, a sample of tank milk was analyzed regarding ESBL-E.coli load. To identify risk factors for higher fecal ESBL-E.coli prevalence, data were collected via questionnaire including general data, data on calving management, feeding and rearing of the calves, farm hygiene management, feeding, milking and dry off management of the cows and antibiotic treatment of the whole herd. Data were statistically analyzed via logistic regression and Spearman's correlation.

Results: The prevalence of ESBL-E.coli in feces of young calves was 63.5 % (95%CI: 57.6-69.4). However, in tank milk we could not detect any ESBL-E.coli. Moreover, there was no correlation between herd size and prevalence of ESBL-E.coli. In 67% of the dairies, waste milk was fed to the calves. Statistical analysis revealed that there is a relationship between waste milk feeding and occurrence of fecal ESBL-E.coli in young calves ($P < 0.05$). Furthermore, in herds feeding milk replacer exclusively the prevalence of ESBL-E.coli in calves was decreased ($P < 0.05$). Acidifying or pasteurization of waste milk did not reduce the occurrence of ESBL-E.coli. Cows of almost all herds were dried off via antimicrobial treatment and in 50% of the dairies, every clinical case of mastitis was treated with antibiotics.

Conclusions: Feeding waste milk is a risk factor for occurrence of ESBL-E.coli in young calves. Dry cow management and treatment of mastitis mostly include antimicrobial treatment. Therefore, high amounts of waste milk are produced. To reduce waste milk feeding to calves resp. to minimize the contamination of the environment, new concepts of dry off management (selective dry off therapy) and research data on treating mastitis (high self-recovery) should be implemented at dairy farms. Prospectively, waste milk feeding to calves should be legally banned.

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61.

Effects of glyphosate residues and varying amounts of concentrates in the rations on performance and blood parameters in fattening bulls

Einflüsse von Glyphosatrückständen und variierenden Kraftfuttermengen in den Rationen auf Leistungs- und Blutparameter in Mastbullen

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Glyphosate (GLY) is one of the most used active substances in broadband herbicides in agriculture worldwide. Therefore, GLY-contaminations in ruminant feed are common and consequently leading to oral exposure (1). Putative GLY effects on health and performance of fattening bulls might be influenced by varying concentrate feed proportions. Therefore, the present study investigated the effects of GLY-contaminated feed in combination with varying amounts of concentrates (C) on parameters associated with performance, energy metabolism and liver health in fattening bulls.
Methods: In a 15-week feeding trial, 47 German Holstein bulls (body weight (BW) 392 ± 60 kg, age 332 ± 29 days; mean ± SD) were assigned to groups fed with either GLY-contaminated (GLY groups) or control (CON groups) rations. The roughage part (79% maize silage, 21% straw based on dry matter (DM)) was fed ad libitum and supplementary low (1kg/animal/day, LC) or high (HC) amounts of C provided by automatic feeding stations. In HC diets, C was increased from 2.5kg /animal/day in the first two weeks to 5kg/animal/day for the following period. During feedstuff production, a part of wheat and peas was treated with Roundup Record® containing GLY as active substance in pre-harvest application according to the regulation (EC) No. 396/2005. The GLY-treated wheat and peas were fed as parts of roughage (wheat straw) and concentrates (wheat kernels and peas) to GLY groups, while CON groups received the respective non-contaminated part of wheat and peas. GLY concentrations in feed were measured by an accredited laboratory. Animals were weighed weekly, whereas daily water intake and DM intake (DMI) were recorded continuously by weighing troughs. Jugular vein blood samples were collected at the beginning of the trial, after seven and after 15 weeks. Serum concentrations of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrat (BHB), cholesterol, triglycerides (TG), total bilirubin, albumin, total protein (TP) and activities of aspartate aminotransferase (AST), alkaline phosphatase (AP), γ-glutamyltransferase (GGT) and glutamate dehydrogenase (GLDH) were determined photometrically. For calculations and statistical analyses, individual intakes were averaged over three week intervals. C, GLY, time (t) and their interactions were set as fixed factors (PROC MIXED, SAS v9.4), individual animal as random factor and time as repeated measure specified within the subject animal. If groups differed significantly ($p<0.05$) in a parameter at the first sampling point, the respective value was used as covariate.
Results: Average daily GLY exposure was 54.4 mg/d (GLYHC), 80.6 mg/d (GLYLC), 0.6 mg/d (CONHC) and 0.8 mg/d (CONLC). BW and DMI was higher in HC groups during the course of the trial ($pC*t<0.05$), while water intake varied over time irrespective of treatment ($pt<0.01$). In HC groups, serum concentrations of NEFA ($pC*t<0.05$) and total bilirubin ($pC*t<0.01$) decreased over time. Glucose levels were temporarily elevated in HC groups during the course of trial ($pC*t<0.01$). BHB, TP ($pC*t<0.01$, $pGLY*t<0.05$), cholesterol ($pC*GLY*t<0.05$) and TG (pC*t, $GLY*t<0.05$) levels were affected by C, GLY and time in an interactive manner. Albumin as well as activity of GLDH showed higher levels in HC groups ($pC<0.05$), whereas AP ($pt<0.01$) and AST ($pGLY*t<0.05$) activity varied inconsistently over time. GGT activity remained unaffected by any treatment
Conclusions: Under applied feeding conditions, C and time affected most investigated parameters. In contrast, GLY did not induce adverse effects on DMI, water intake and BW or on most blood parameters. This corresponds to results in German Holstein cows (2). GLY influences appeared to be significant for AST, BHB, cholesterol, TG and TP. These findings were most likely incidental, since high variation was observed within the experimental groups. Therefore, the biological relevance of these results remains questionable and further analyses are needed.

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62.

Investigations on the transfer of quinolizidine alkaloids from blue sweet lupins (*Lupinus angustifolius*) into the milk of dairy cows – a pilot study

Untersuchungen zum Transfer von Quinolizidinalkaloiden von blauen Süßlupinen (Lupinus angustifolius) in die Milch von Kühen – eine Pilotstudie

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Blue sweet lupine (BSL) seeds are used as an alternative protein source to substitute soybean or rapeseed meal in dairy cow nutrition (1,2). Despite having generally low (0.02 to 0.5%) contents of quinolizidine alkaloids (QAs) compared to bitter lupins (5 to 8%), QA contents in sweet lupins can vary depending on cropping, environmental conditions and backcrossing with wild (bitter) varieties. In humans and animals, QAs have an inhibitory effect on the acetylcholine receptor. Acute toxicity symptoms in humans are usually described as anticholinergic syndrome, often also affecting the cardiovascular and digestive systems (3). The transfer of QAs from feed into milk has been hypothesized due to their weak basic nature and from indirect evidence, but no direct experimental data is currently available (3). Thus, we conducted a pilot study with lactating cows to evaluate the possible transfer of QAs from BSL into the milk of dairy cows.

Methods: Four Holstein-Friesian dairy cows (3 multiparous and 1 primiparous) in mid lactation (100-150 lactation days) with an average milk yield of 32.0 ± 4.7 kg d-1 were used in this study. Cows were kept in one group in an open barn stable with free access to water and were milked twice daily. Isocaloric diets were formulated and offered as partial mixed ration in feeders with built-in scales allowing for the control of daily feed intake. After a one-week adaptation period (AP), rapeseed meal (383 g kg-1 CP) was partially replaced (1 kg w/w 88% DM) with BSL (Lupinus angustifolius var. Boregine (293 g kg-1 CP). The BSL were offered in two meals per day separately from the diet to ensure complete consumption by each cow. The low dose BSL diets were fed for one week (Exp1) followed by a 10-day depuration period 1 (Dep1) with BSL-free diets. Subsequently, two kilograms of rapeseed meal were replaced by BSL (w/w) for another 7-days (Exp2) followed by a 14-day depuration period (Dep2) of BSL-free feeding. During the entire experimental period, milk samples were taken twice daily and regularly analyzed for milk fat, protein, lactose, urea, and somatic cell counts following standard protocols. Known QAs occurring in Lupinus species (i.e. sparteine, lupanine, 13-α-hydroxylupanine, isolupanine, angustifoline, lupinine, anagyrine, multiflorine) in feed and milk samples were quantified using an in-house validated, novel LC-MS/MS method. Based on feed and milk QA contents, feed intake and milk yield, a three-compartment toxicokinetic model was developed in Python programming language to evaluate the feed-to-food transfer of the above-mentioned QAs. Transfer rates (TR) and half-lives for QA elimination during Dep1 and Dep2 were estimated using numerical simulations and from model equations, respectively. Differences between periods for DM intake and milk yield were analyzed using the Repeated Measures ANOVA in SAS 9.4.

Results: The sum QA content in BSL ranged between 0.18 - 0.20% DM. No adverse effects of lupin feeding were observed during the entire period. Average DM intake did not differ significantly between AP, Exp1, Dep1, Exp2 and Dep2, respectively, despite a general numerical decline. Accordingly, milk yield declined over the course of the experiment to 29.1 ± 2.0 kg d-1 (period effect $P<0.05$). Already with the administration of 1 kilogram of lupins, a transfer into the milk could be demonstrated. Individual QAs show transfer rates into the milk in the order of 1 to 5 revealing high animal-specific variations in TR. Isolupanine displayed higher TR than lupanine or hydroxylupanine, respectively. Similarly, daily variation in quantitative excretion patterns suggest metabolic processes including detoxification of or biotransformation among QAs. In contrast to TR, milk excretion half-lives of individual QAs did not differ significantly and were in the order of <1 day.

Conclusions: The present pilot study shows that a transfer of lupin QAs from feed to food of animal origin is possible, even when sweet lupin varieties are fed. Further data are required to quantitatively evaluate the transfer and biotransformation of QAs during their transition from feed into milk using a larger number of cows and accompanying *(in vitro)* studies.

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63.

Effects of ensiling conditions and silage quality on the degradation of pyrrolizidine alkaloids from Senecio spp. in grass silage

Effekte der Silierbedingungen und Silagequalität auf den Gehalt an Pyrrolizidinalkaloiden von Senecio spp. in Grassilage

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Besides other Senecio spp., abundances of common ragwort (S. jacobaea) and eastern groundsel (S. vernalis) have increased on fields used for forage production in several countries.Since Senecio spp. can contain substantial amounts of pyrrolizidine alkaloids (PA) with hepatotoxic and carcinogenic properties (1), they are considered a health risk for both animals and humans. Previous experiments with laboratory-scale silages showed that molasses addition prior ensiling beneficially affected the degradation of the PA senecionine and seneciphylline from S. vernalis (2). To follow up we hypothesized that ensiling conditions significantly affect microbial PA metabolism and PA contents in silages. Thus, we conducted a laboratory-scale ensiling experiment, provoking good and poor ensiling conditions for grass silages contaminated with either S. jacobaea or S. vernalis.

Methods: Silages were prepared in 1.5 L Weck® jars in a multifactorial design in quadruplicates for each treatment combination. Treatments were two different DM levels obtained by either pre-wilting or no pre-wilting, two contaminations with 3% (on DM basis) of either S. jacobaea or S. vernalis and four different ensiling treatments. Treatments were: 1) untreated control, 2) addition of heterofermentative Lactobacillus buchneri strain LN4637at 7.3 × 104 cfu/gplus 30 g/kg molasses, 3) addition of homofermentative lactobacilli at 7.3 × 104 cfu/gplus 30 g/kg molasses, or 4) addition of 10 g sand. Each silage was individually prepared in a plastic trough and thoroughly mixed before being filled into the glass jar, sealed and stored at ambient temperature for 90 days. After 90 days weight loss was recorded and samples were taken for analyses of DM content, pH, volatile fatty acids (VFA) and concentration of PA. VFA were detected by GC, PA and their N-oxides were analyzed by LC-MS/MS. Data were analyzed by ANOVA in a multifactorial design followed by Tukey-Kramer post hoc test using SAS.

Results: After 90 days of storage DM averaged 30.8% for the not pre-wilted grass silage and 35.4% for the pre-wilted grass silage. Likewise pH differed between the two DM levels ($P<0.001$), being 4.2 and 4.4, respectively. Ensiling treatments affected VFA formation in the silages, as acetic acid was highest with the L. buchneri strain LN4637inoculum ($P<0.001$), and sand contamination resulted in increased n-butyrate content ($P<0.001$). Silages supplemented with S. vernalis displayed higher contents ($P<0.001$) of total PA (8132 µg/kg; fresh matter basis) than silages with S. jacobaea (923 µg/kg). The PA-composition in silages reflected the Senecio spp. added, meaning that in S. jacobaea contaminated silages the most abundant PA were erucifoline, followed by retrorsine, while in S. vernalis contaminated silages the most abundant PA were senecionine and/or its isomers, followed by senkirkine. Dry matter content and ensiling treatments affected contents of several PA. The inoculation with both homofermentative and heterofermentative lactobacilli plus molasses resulted in decreased concentrations of erucifoline and senecionine and its isomers in comparison to control and sand treatments ($P<0.05$). Inoculation with L. buchneri strain LN4637decreased retrorsine in comparison to control and sand treatments ($P<0.001$). Contrastingly, contents of senkirkine remained unaffected by ensiling treatment.

Conclusions: The present study showed that ensiling conditions affect the PA disappearance during fermentation, thus influencing the final total PA content. Using bacterial inoculants and molasses could help to decrease the content of several PA in silage. However, the otonecine type PA senkirkine remained unaffected and the total PA content was still high for certain contamination profiles.

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64.

Occurrence of mycotoxins and other fungal metabolites in pastures in Austrian dairy farms

Vorkommen von Mykotoxinen und anderen Pilzmetaboliten auf Weiden österreichischer Milchviehbetriebe

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Grasses and grass-legume mixtures are fundamental feed sources for the dairy production, which uses these as fresh pastures as well as conserved and stored forages. Therefore, pastures could contribute to the mycotoxin contamination in feeds used in dairy production. Data on mycotoxin occurrence in pastures are limited. This study investigated the mycotoxin occurrence in pastures in Austrian dairy farms.

Methods: Pastures samples were collected during April - October 2019 from 18 dairy farms located in Styria (n = 8), Lower Austria (n = 5) and Upper Austria (n = 5) with an altitude between 235 – 1340 m.a.s.l. Per farm, 30 incremental samples from a grazed paddock were pooled, vacuum-packed, and stored at -20 °C. The sampled pastures contained mixtures of Gramineae such asDactylis glomerata, Lolium perenne, Poa pratensis, and Alopecurus pratensis and Leguminosae such as Trifolium spp. and Medicago sativa. We identified major botanical species in the pooled samples and, visually, Gramineae dominated the pastures but the exact proportions of individual species were not determined. The samples were analyzed via LC-MS/MS (Spectrum 380®). The concentration values are presented on a dry basis in parts per billion (ppb). The average dry matter content of pasture samples was 23.28 ± 6.33%.

Results: In total, 58 different mycotoxins and metabolites were detected, ranging from 8 to 48 compounds per sample (mean = 25). Apart from 100% prevalence of some unspecific metabolites [e.g., cyclo(L-Pro-L-Tyr), rugulosovin, brevianamide F, and tryptophol], the Fusarium metabolites culmorin, moniliformin, and aurofusarin were the most frequent contaminants. Other "emerging" mycotoxins (e.g., enniatins and beauvericin) were also detected in high frequency. Nivalenol and altersetin were detected in about 80% of the samples, while contaminations with zearalenone, deoxynivalenol and ergot alkaloids were less frequent, with average concentrations of 29.59 ± 44.26, 305.99 ± 280.88 and 162.79 ± 191.16 ppb, respectively. Aflatoxins, T-2 toxin, HT-2 toxin, ochratoxins, and fumonisins were not detected. Cyclo (L-Pro-L-Val), sydowinin A and cyclo (L-Pro-L-Val) were the metabolites with the highest concentrations (on average): 2192.95 ± 1002.82, 719.25 ± 1401.4 and 498.45 ± 347.50 ppb, respectively.

Conclusion: Although the analysed pastures did not show high contamination levels of regulated mycotoxins, a broad range of emerging mycotoxins was evident. The recent study underlines pastures as a source of multi-mycotoxin contamination entering the feed chain.

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65.

Preliminary data on the interaction of microplastics with the ruminal fermentation *ex vivo*

Erste Daten zur Interaktion von Mikroplastik mit der ruminalen Fermentation ex vivo

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Microplastics are estimated to accumulate in European agricultural soils in a range between 63,000 to 430,000 t/a (1). Furthermore, these materials have recently also been detected in human stool samples, thereby proving their circulation within the food chain (2). To the best of our knowledge, no data is yet available on the fate of such materials in livestock digestive systems. As a first step, this study examined the interaction between different chemical microplastic species supplied in different average particle size distribution at varying dosages on the ruminal fermentation *ex vivo*.

Methods: The experiments were performed with the Hohenheim Gas Test according to published standard procedures (3). Rumen-fistulated rams served as donor animals for rumen fluid, which was always collected before the morning feeding. The microplastic species polylactide (PLA), polyhydroxy butyric acid (PHB), high-density polyethylene (HDPE), polyvinyl chloride (PVC) and polypropylene (PP) were applied in different particle size ranges (<125 µm and 125-500 µm, respectively; PP was only available at 125-500 µm). These materials were weighed into incubation cylinders at respective total amounts of 0, 0.5, 5, 35 and 70 mg thereby representing 0%; 0.2%; 2%; 14% and 28% of weighed dry feed mass within each cylinder, respectively. Either 250 mg of dried barley or hay, respectively, were supplied as fermentable feed materials. Each treatment variant (microplastic species * particle size range * dose * feed) was incubated during three consecutive runs (24 h, 39 °C, 1 rpm), respectively. At the timepoint of submission, first data from the incubations of all treatment variants in the presence of barley was available. This comprised cumulative microbial gas production, pH, and volatile fatty acids after 24h of incubation. Statistical analysis comprised multi-factorial ANOVA (plastic source, particle size, dose) including interactions.

Results: In general, the cumulative gas production as well as volatile fatty acid concentrations after 24h showed in any case numerically decreased values compared to the untreated negative control. However, this difference could not be analyzed by statistical analysis due to the yet limited size of this preliminary dataset. The cumulative gas production over 24h of incubation was significantly ($P = 0.03$) affected by the plastic source with PHB showing significantly increased values compared to HDPE and PP, whereas PLA and PVC showed no difference to each other or any other source. Furthermore, isobutyrate, isovalerate and valerate showed significant differences or tendencies with respect to plastic source and/or size distribution, respectively (isobutyrate: source $P = 0.05$, size $P = 0.08$; 0.42, 0.41, 0.40, 0.39 and 0.35 mM/L for PP, PLA, PVC, HDPE and PHB; 0.40 and 0.38 mM/L for <125 and 125-500 µm; isovalerate: source $P = 0.08$; 0.50, 0.47, 0.46, 0.44 and 0.41 mM/L for PP, PVC, PLA, HDPE and PHB; valerate: source $P = 0.04$, size $P = 0.05$, size*dose $P = 0.09$; 1.02, 0.98, 0.97, 0.94 and 0.89 mM/L for PP, PVC, PLA, HDPE and PHB; 0.97 and 0.94 mM/L for <125 and 125-500 µm). The pH after 24 h was not affected by the plastic supplementation whatsoever.

Conclusion: In conclusion, our first data points towards reduced microbial activity in the presence of microplastics irrespective of source or dose when comparing it to a non-supplemented negative control. This must not necessarily have been due to specific interactions with microbes but could have also represented a general dilution effect. However, the specific effects of plastic sources at different particle size distribution on the production of iso-volatile fatty acids and valerate suggests an interaction with specific microbial metabolic pathways. These preliminary findings should be reproduced by completing our dataset and by further investigating the adaption of the ruminal microbiome on the proteomic and metabolomic level.

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66.

The effects of age, gender, breed and feed additives on ileal histomorphology and gene expression of broilers

Die Auswirkungen von Alter, Geschlecht, Rasse und Futterzusätzen auf die Histomorphologie des Ileums und die Expression immunrelevanter Gene im Broiler

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The intestinal morphology and immune system of broilers may be affected by internal and external factors. This study investigated the effect of age, breed, gender, a probiotic and phytobiotic supplementation and their interactions on histomorphology, goblet cell numbers, gene expression related to epithelial barrier function and inflammatory markers of the ileum in broilers. **Methods:** A total of 2,880 one-d-old male (M) and female (F) broiler chicks from two different breeds, Ross-308 (RS) and Cobb-500 (CB), were randomly allocated to 72 pens. Broilers were offered 3 experimental diets including a standard wheat-soybean based diet without (control) or with supplementation of either a probiotic (2.4 x 10^9 CFU/kg diet; *Bacillus subtilis*, DSM32324 and DSM32325 and Bacillus amyloliquefaciens, DSM25840) or a phytobiotic (165 ppm procyanidins and 585 ppm polyphenols) product. At day 7, 21, and 35, one chicken per pen was sacrificed to collect the midpoint of the ileum. Data were subjected to ANOVA using GLM procedure with a 3 (age) × 2 (breed) × 2 (gender) × 3 (dietary treatment) factorial arrangement of the main factors. **Results:** From day 7 to 35, villus height (VH) and crypt depth (CD) increased by 85% and 70%, respectively while villus width (VW) only increased by 28% from day 7 to 21 ($P < 0.05$). Females showed longer villi than males ($P < 0.05$). The other main factors had no impact on VH, CD and VW ($P > 0.05$). The total goblet cell numbers per villus increased by age while it was higher in RS and F than CB and M, respectively ($P < 0.05$). By the Alcian Blue and Periodic Acid Schiff staining method, the mixed and acidic goblet cells were differentiated. The number of acidic goblet cells increased from day 7 to 35, while the mixed goblet cell numbers increased only from day 7 to 21 ($P < 0.05$). Increased number of mixed goblet cells was also observed in F compared with M ($P < 0.05$). There was no significant effect of dietary treatments on either total, acidic or mixed goblet cells number per villus ($P > 0.05$). The normalized absolute gene expression was affected by age, dietary treatment and breed ($P < 0.05$). The gene expressions of interleukin (IL)-1, 2, 4, 6, 8, 10, 12, 17, 18, as well as TNF-α, IFN-γ and TGF-β increased from d 7 to 21 by 2.5, 1.5, 2.7, 0.8, 1.2, 1.9, 2.0, 1.2, 1.7, 1.3, 1.6 and 0.2 log10 copies/ng RNA, respectively but they decreased by 1.6, 1.3, 2.1, 1.1, 1.4, 1.1, 1.2, 1.8, 1.3, 1.6, 2.0 and 2.6 log10 copies/ng RNA from d 21 to 35, respectively ($P < 0.05$). Furthermore, the gene expression of all the interleukins, TNF-α, IFN-γ and TGF-β was significantly different between d 7 and 35 ($P < 0.05$). The interaction between age and treatment was significant for the expression of IL-18 and TNF-α, while the interactions between age and breed as well as treatment and breed were significant for IFN-γ and IL-10 expressions, respectively ($P < 0.05$). CB showed higher expression of IL-4, IL-6 and TNF-α (by 0.09, 0.22 and 0.10 log10 copies/ng RNA, respectively), while RS displayed a greater IFN-γ expression (by 0.08 log10 copies/ng RNA; $P < 0.05$). The gene expression of mucin-related MUC2 and tight junction-related CLDN5 increased from day 7 to 21 by 2.0 and 2.3 log10 copies/ng RNA, respectively and then decreased by 1.7 and 1.8 log10 copies/ng RNA ($P < 0.05$). The age x breed and age x treatment x breed x gender interactions were significant for the CLDN5 gene expression ($P < 0.05$). **Conclusions:** In conclusion, age had significant impacts on almost all the variables measured in the ileum, while the nutritional treatments had almost no impact on the investigated variables. A few observed significant impacts of breed and gender on the variables measured did not show any systematic biological pattern.

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67.

The effect of birth weight and age on jejunal tight junction mRNA abundance and cellular proliferation during the suckling period in male piglets

Der Einfluss von Geburtsgewicht und Alter auf Transkriptabundanz von Tight Junction Proteinen und Zellproliferation im Jejunum von männlichen Saugferkeln

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The jejunum is an important gut section for nutrient absorption that undergoes rapid growth and development during the neonatal phase in suckling piglets. It has been previously reported that this development is impaired in low birth weight (L) piglets (1), with reduced intestinal tight junction protein (TJP) mRNA abundance (2) and cell proliferation (3) observed in comparison to their normal birth weight (N) piglets. Whether these differences remain during the entire suckling phase is unclear. We hypothesize that TJP mRNA abundance and jejunal cell proliferation are lower in L compared to N piglets during the pre-weaning phase. **Methods:** In this study, male L (0.8-1.2 kg, n = 72) and N (1.4-1.8 kg, n = 72) littermates born to gilts were selected at birth (day of life (d) 0). Litters were standardized to 12 piglets on d 1, piglets were given access to creep feed from d 14, and suckled by their respective dam for the length of the study. On d 5, 12, and 26 random sub-groups (n = 48; 24 / birth weight group) of piglets were euthanized. Two hours before euthanasia piglets were i.p injected with 12 mg/kg body weight of bromo deoxyuridine (BrdU) to measure cellular proliferation. At euthanasia, piglets were weighed and jejunal tissue was sampled and frozen to analyze tight junction: Claudin-4, Zonula Occludens-1, 2 (ZO-1, ZO-2), Occludin, and cellular proliferation marker: Proliferating-Cell-Nuclear-Antigen (PCNA), mRNA abundance. The incorporation of BrdU into replicating DNA and TJP localization was measured using immunohistochemistry (IHC). Data was analyzed using the MIXED procedure of SAS. Least square means were separated using the Tukey test ($P < 0.05$). **Results:** Birth weight group comparisons at d 5 (L vs N; 1.74 kg vs 2.28 kg), d 12 (L vs N; 3.24 kg vs 3.98 kg), and d 26 (L vs N; 6.30 kg vs 7.55 kg) showed L piglets remained lighter than N littermates ($P < 0.05$). No differences in mRNA abundance or BrdU incorporation was observed. A comparison of age levels showed that at d 12 both L and N piglets were heavier than at d 5 ($P < 0.05$). However, the abundance of ZO-2 mRNA was significantly ($P < 0.05$) and ZO-1 tended ($P < 0.10$) to be lower in L piglets only, at d 12 compared to d 5. No other differences were observed. A comparison of d 26 piglets showed both L and N were heavier ($P < 0.05$), and the abundance of Claudin-4, Occludin, ZO-1, ZO-2 and PCNA ($P < 0.05$) mRNA was lower in both L and N piglets, compared to d 12. Additionally, the area of BrdU positive cells was larger at 26 d in the crypt and the villus area ($P < 0.05$) compared to 12 d, in both L and N piglets. Immuno-histochemical staining confirmed TJP localization between enterocytes in the crypt and villus area **Conclusion:** Our results obtained in male piglets seem not to confirm previous results (2, 3) on the association between L birth weight and reduced jejunal TJP mRNA abundance and cell proliferation. However, the d 5 to 12 comparison does indicate that only L piglets undergo a reduction in the abundance of the two critical regulators of TJ assembly, ZO-1 and ZO-2. These data will be supplemented by Western Blot and gut permeability investigations in future studies.

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68.

Effect of thymol on electrophysiological parameters in the porcine jejunum and colon *ex vivo*

Effekt von Thymol auf elektrophysiologische Parameter im porcinen Jejunum und Colon ex vivo

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The effect of plant bioactive ingredients on the digestion of animals is of great interest. Some of these phytoactive compounds, such as thymol, are known to activate specific channels of the transient receptor family (TRP), triggering various signalling cascades. TRPV3 channels are permeable to various cations such as Ca^{2+} and Na^{+} and a role in ion transport has been shown in the rumen [1,2,3]. It was the aim of this study to investigate the expression of this channel in pig jejunum and colon and to study the electrophysiological effects of the TRPV3 channel agonist thymol in these tissues.

Methods: Jejunum and colonic tissue (both from the middle segment) was taken from 10 pigs of ~10 weeks. Expression of TRPV3 was investigated by qPCR, Western blot and immunohistochemistry (IHC). Fresh tissues were stripped and mounted in Ussing chambers (Ringer with 95% O_2/5% CO_2) to determine the short-circuit current (Isc) and conductance (Gt). Statistical comparisons were evaluated using either a t-test or rank sum test and the data are presented as mean values and SEM.

Results: After an equilibration time of 45 minutes, addition of 1 mmol·l-1 thymol to the tissue on both sides resulted in abrupt changes in Gt and Isc that could not be observed in response to the solvent ethanol (0.1%). In both tissues, the Gt rose significantly. Conversely, the Isc response depended on the sample. In the jejunum, half of the tissues responded with an increase (UP) in Isc by ΔIsc = 0.22 ± 0.04 µeq·cm-2·h-1 to an end value of 1.33 ± 0.28 µeq·cm-2·h-1 (P = 0.004, n/N = 9/4). In the other 9 jejunal tissues, a decrease (DO) by 0.17 ± 0.06 µeq·cm-2·h-1 to -0.08 ± 0.17 µA·cm-2 (P = 0.004, n/N = 9/4) was observed. The change in ΔGt was similar at 2.69 ± 0.99 mS·cm-2 (UP; P = 0.020) and 1.94 ± 0.61 mS·cm-2 (DO; P = 0.020) to a total mean of 22.23 ± 4.21 mS·cm-2 for the jejunum (P < 0.001, n/N=18/8). Similar effects were observed in the colon. In the colonic UP group, Isc increased by ΔIsc = 0.20 ± 0.04 µeq·cm-2·h-1 (P = 0.016, n/N = 7/4) to a mean value of 0.74 ± 0.30 µeq·cm-2·h-1, while in the DO group, Isc decreased by ΔIsc = 0.22 ± 0.10 µeq·cm-2·h-1 to a final Isc of 0.84 ± 0.28 µeq·cm-2·h-1 (P < 0.001, n/N = 11/7). The Gt of the UP group increased by 2.36 ± 0.74 mS·cm-2 (P = 0.016) and of the DO group by 0.84 ± 0.37 mS·cm-2 (P = 0.042), with mean Gt of all colonic tissues at 22.05 ± 1.05 mS·cm-2 (P < 0.001, n/N=18/10). Expression of TRPV3 could be confirmed via Western blot and IHC staining in both tissues, primarily localized to the apical membrane of the epithelium. Conversely, any staining of the crypts and the basal layers of the epithelium was weak, suggesting a function in absorption. On the level of mRNA, only colonic samples were positive for TRPV3, possibly reflecting increased turnover of epithelial cells with formation of new protein by this tissue.

Conclusion: While the increase in Gt in response to thymol may indicate either an opening of a paracellular or a transcellular pathway, only active transcellular transport of ions can explain the simultaneous increase in Isc observed in roughly half of the tissues from both epithelia. In conjunction with the molecular biological data demonstrating the expression of TRPV3 on the protein level, the most likely explanation is that thymol opened TRPV3 channels with an increase in the transcellular absorption of Na^{+}. Concomitant influx of Ca^{2+} with activation of chloride secretion may have augmented this response. A secretion of K^{+} by TRPV3 or other potassium conductances of the epithelium should explain part of the decrease to negative Isc values observed in a number of epithelia. In conjunction with molecularbiological data confirming expression of TRPV3, we conclude that this channel may be involved in transport of ions such as Na^{+}, NH^{4+} and Ca^{2+} across the jejunum and colon of pigs. Given the action of plant terpenoids on TRPV3, implications for novel feeding strategies abound.

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69.

Effects of phytogenic compounds on electrophysiological parameters and barrier integrity in the porcine jejunum *ex vivo*

Effekte phytogener Wirkstoffe auf elektrophysiologische Parameter und Barriereintegrität im Jejunum von Schweinen ex vivo

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Phytogenic ingredients are increasingly used in the feeding of animals with different effects on growth, health or feed efficiency. However, the specific modes of action of many of these ingredients have not yet been sufficiently researched. Some of these phytogenic substances can activate specific channels of the transient receptor potential (TRP) family. These channels can also be found in the epithelium of the intestinal tissue, but the function of these channels is still largely unknown. For example, a release of serotonin after activation of TRPA1 in enterochromaffin cells was suspected [1], causing subsequent anion secretion. Recent studies showed distinct pattern in ion transport after the application of different TRPA1 agonists [2]. Therefore, the aim of the present study was to investigate the effect of three TRPA1 agonists on epithelial barrier and electrophysiological parameters and to gain insight into the involved cascades of action.

Methods: Jejunal epithelial preparations from 12 pigs were mounted in Ussing chambers and incubated with a standard chloride and bicarbonate-containing solution either without additions (CON) or with serosal addition of 10 µM granisetron, 10 µM ketanserin and 10 µM SB204070 as serotonin receptor antagonists (5HT-X) or with bilateral addition of 10 µM indomethacin as prostaglandin synthesis inhibitor (PGE2-X). Another set of epithelia was incubated in chloride and bicarbonate-free solution containing ethoxyzoleamide as carbonic anhydrase inhibitor (ANION-X). After these pre-treatments, ethanol or 1 mM of either cinnamaldehyde (CIN), eugenol (EUG) or citral (CTA) was added on the mucosal side to each group. Short-circuit current (Isc) and conductivity (Gt) were measured over three 45-min periods in which mannitol flux rates were measured to estimate paracellular integrity. Pro-secretory effects of the applied TRPA1 agonists were assessed based on maximum increases of Isc and Gt. Mannitol flux rates were arithmetically pooled over the three flux periods before analysis. The statistical analysis was performed with the software SPSS 26 and a linear mixed model with pre-treatment, TRP agonist, as well as their interaction as fixed factors and animal as random factor.

Results: Addition of CIN and CTA, but not EUG, caused an increase in Isc in CON and 5HT-X ($P < 0.001$) with no difference between CON and 5HT-X. However, this increase was not observed in PGE2-X and ANION-X. In addition, a temporary drop in Gt (over ~10 min) was observed in CON, 5HT-X and PGE2-X after the addition of CIN ($P < 0.05$). Interestingly, this electrophysiological effect could not be observed in EUG. However, the addition of EUG resulted in increased mannitol flux rates in CON ($P < 0.01$), which was not observed for CIN and CTA. Furthermore, increased mannitol flux rates in PGE2-X was noticed in CIN and EUG. No differences in flux rates could be found in the 5HT-X group.

Conclusion: The increased Isc after CIN or CTA addition suggests TRP-triggered chloride and/or bicarbonate secretion mediated by the prostaglandin pathway because these effects could not be observed after blocking prostaglandin synthesis and in solution free of chloride and bicarbonate. The involvement of serotonin-mediated anion secretion through the activation of TRPA1 could not be confirmed in this trial. The increased mannitol flux rates after CIN addition in PGE2-X solution support the epithelial protective effect of prostaglandins, but it remains unclear why this effect was not observed for CTA treatment. A different mechanism could be responsible for EUG because higher mannitol flux rates were observed in CON and PGE2-X after EUG addition. It should be noted, however, that relatively high concentrations of 1 mM TRP agonists were used in this experiment.

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70.

The effect of butyrate on transport of Ca^{2+} and Na^{+} through the bovine TRPV3 channel.

Der Effekt von Butyrat auf den Transport von Ca^{2+} und Na^{+} durch den bovinen TRPV3 Kanal.

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Hypocalcaemia around calving is a serious problem in cows. Many studies of ruminal transport have shown that short-chain fatty acids (SCFAs) stimulate transport of Ca^{2+} but the mechanism is unknown. Previous attempts *in vivo* and *in vitro* have failed to identify the classical epithelial Ca^{2+} channels TRPV5 and TRPV6 (1). Recently, TRPV3 has emerged as a candidate for the ruminal transport of cations, with substrates including Ca^{2+}, Na^{+}, and NH^{4+} (2, 3). The purpose of the present study was to investigate whether the bovine representative of TRPV3 (bTRPV3) can be stimulated by application of the SCFA butyrate at pH 7.4 and pH 6.4.

Methods: Measurements of intracellular calcium concentration ($[Ca^{2+}]i$) were performed with HEK-293 cells that were either transfected with the bTRPV3- or with the empty pcDNA5/TO (control) vector for 1-2 days. Cells were initially superfused at pH 6.4 with chloride Ringer (NaCl) that contained sodium gluconate which was subsequently replaced by an equimolar (30 mmol · L-1)amount of sodium butyrate (NaBu). Cells were loaded with fura-2 AM for intracellular calcium fluorescence imaging measurements using an Olympus CellSens Dimension system.

The Na+ conductance was determined in bTRPV3- or empty pIRES2-AcGFP1 (control) transfected HEK-293 cells using the whole-cell configuration of the patch-clamp technique. The pipette was filled with calcium-free sodium gluconate solution, whereas bath solutions were the same as in calcium imaging experiments but adjusted to both pH 7.4 and 6.4. Values are given as means ± SEM and nonparametric tests were used to detect differences.

Results: In calcium imaging experiments, bTRPV3 and control cells showed similar $[Ca^{2+}]i$ values (bTRPV3: 30 ± 6 nmol · L-1; control: 21 ± 5 nmol · L-1; $p = 0.2$) in NaCl (pH 6.4), suggesting efficient mechanisms for regulating Ca^{2+} influx and efflux. Exposure to NaBu (pH 6.4) led to a significant rise of $[Ca^{2+}]i$ in both groups, with the increase in bTRPV3 (by 143 ± 16 nmol · L-1 ; n/N = 64/5; $p \leq 0.001$) significantly higher ($p \leq 0.001$) than that in controls (by 42 ± 4 nmol · L-1; n/N = 92/5; $p \leq 0.001$). After wash out, $[Ca^{2+}]i$ returned to a value close to the original level with no differences observed between the two groups ($p = 0.9$).

In whole-cell experiments, currents clamped at +100 mV and -120 mV were visibly affected by application of NaBu at pH 7.4, although values did not reach significance. Wash out resulted in a return to the original level. A switch from pH 7.4 to 6.4 in NaCl had no effect on whole-cell currents (bTRPV3: $p = 1.0$; control: $p \geq 0.3$). However, a switch to NaBu (pH 6.4) led to a significant rise in current amplitude in bTRPV3 at both +100 mV by 76 ± 19 pA · pF-1 (n = 12; $p \leq 0.001$) and at -120 mV by 26 ± 17 pA · pF-1 ($p = 0.003$), with partial washout after return to NaCl (pH 6.4) and finally NaCl (pH 7.4). In contrast, any effects of NaBu (6.4) in controls did not reach significance with current amplitude numerically decreasing at +100 mV by -7 ± 8 pA · pF-1 (n = 17; $p = 0.7$) and at -120 mV by 2 ± 2 pA · pF-1 ($p = 0.8$).

Conclusions: The calcium imaging experiments showed that butyrate stimulates influx of Ca^{2+} through bTRPV3 channels, while whole-cell data showed a stimulatory effect of butyrate on Na^{+} transport. The effects were visible at pH 7.4 and significant at pH 6.4 arguing for a role of intracellular protons in the signaling cascade. Given the known effect of SCFA on ruminal Ca^{2+} transport, these data imply that bTRPV3 plays a significant role in the uptake of this cation. Furthermore, stimulation of Na^{+} transport through bTRPV3 may augment the effects of SCFA on the ruminal Na^{+}/H^{+} exchanger (NHE), with protons extruded via the H^{+}-ATPase. This should play a significant role at low ruminal pH (< 6.4), where the driving force for NHE becomes precarious. Identifying the proteins responsible for uptake of cations in general and Ca^{2+} in particular by the rumen opens new possibilities for ruminant nutrition and healthcare.

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71.

Effects of menthol on epithelial calcium channel expression and functional calcium absorption in the gastrointestinal tract of sheep

Wirkung von Menthol auf die Expression epithelialer Calciumkanäle und die funktionelle Calciumresorption im Gastrointestinaltrakt von Schafen

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Gastrointestinal Ca^{2+} absorption plays a pivotal role in Ca^{2+} homeostasis, but the mechanisms and transporting proteins involved in ruminal Ca^{2+} absorption are not completely clarified. Classical Ca^{2+} channels are not present in the rumen. Previous studies strongly indicate an active transport mechanism, which contributes to a significant part of gastrointestinal Ca^{2+} absorption. Ruminal Ca^{2+} absorption can be stimulated by plant bioactive lipid compounds (PBLC) such as menthol *in vitro*, most likely by the activation of TRPV3 channels [1]. *In vivo* studies revealed increased plasma Ca^{2+} concentrations in cows and sheep after long-term menthol supplementation [2,3]. The present study was conducted to investigate the dose-dependent effect of menthol pre-feeding and the acute menthol stimulation of ruminal and jejunal Ca^{2+} absorption in sheep. Further, the presence and quantitative changes of Ca^{2+} transporting TRP channels were measured to identify the effects of menthol at molecular levels in the gastrointestinal tract. **Methods:** Twenty-four growing Suffolk sheep were divided in three treatment groups and received 0 (control), 80 (PBLC-L) and 160 mg/d (PBLC-H) of a menthol-containing PBLC feed additive. After 28 days of feeding, tissue samples of the rumen and the mid-jejunum were taken and mRNA levels of TRPA1, TRPV3, TRPV5, TRPV6, and TRPM8 channels were analyzed by RT-qPCR. Ca^{2+} transport was measured in Ussing chambers using radio-labeled isotopes to evaluate absorption differences among feeding groups in the rumen and jejunum. To test for acute menthol-induced stimulation of Ca^{2+} absorption, menthol (50 µmol·l-1) was applied to the mucosal side of ruminal and jejunal epithelia. Data were analyzed by a mixed model procedure with respect to linear and quadratic effects of PBLC using polynomial contrasts. **Results:** TRPA1 was present in both rumen and jejunum. TRPV3 expression was restricted to the rumen. Expression of TRPV5 and TRPV6 was detected in the jejunum but not in the rumen. No quantitative changes occurred on the mRNA level between feeding groups in all examined genes in the rumen. In the jejunum, TRPA1, TRPV5 and TRPV6 tended to decrease linearly with increasing PBLC dose. Although passive Ca^{2+} permeation was high, no significant net Ca^{2+} absorption was observable in the jejunum in all groups. In ruminal tissues, net Ca^{2+} transport increased with increasing dose of PBLC in a quadratic manner ($P = 0.043$). After mucosal menthol addition, ruminal tissues reacted with increased net Ca^{2+} absorption with significantly greater stimulation in animals pre-fed with PBLC ($P = 0.038$). No effect of acute menthol addition was detected in jejunum. **Conclusion:** This study underlines the importance of the rumen in gastrointestinal Ca^{2+} absorption. Levels of TRPV5 and TRPV6 mRNA expression were low in the jejunum. The low levels of jejunal TRPV6 are in contrast to previous studies on ovine TRPV6 distribution; however, they fit to the absence of active Ca^{2+} transport in the jejunum in the present study. Long-term PBLC pre-feeding stimulates Ca^{2+} absorption capacity in the rumen in a quadratic manner, but has no effect on the mRNA levels of TRP genes, which may indicate a post-transcriptional influence of menthol. The fact that acute menthol stimulation of Ca^{2+} absorption occurred only in the rumen points towards the involvement of TRPV3 in ruminal Ca^{2+} absorption because this channel was detectable in ruminal but not in jejunal tissue.

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72.

Influence of supplementing menthol-rich plant bioactive lipid compounds on the epithelial barrier in ruminal and jejunal epithelia of sheep.

Einfluss einer Supplementierung von Menthol-haltigen bioaktiven Pflanzenlipiden auf die epitheliale Barriere im Pansen und Jejunum von Schafen.

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Menthol, a plant bioactive lipid compounds (PBLC) stimulates transcellular Ca^{2+} absorption *ex vivo* in the rumen by agonistic binding to transient receptor potential (TRP) channels, which results in elevated Ca^{2+} plasma levels of cows and sheep *in vivo* [1,2]. Furthermore, the PBLC menthol is known to act as an absorption enhancer via the paracellular pathway [3]. It is still unclear whether the observed increase in Ca^{2+} absorption is solely due to the agonistic effect of PBLCs on transcellular transport via TRP channels or whether a menthol-rich PBLC pre-feeding also affects paracellular permeability. **Methods:** Suffolk sheep (n = 24) were equally distributed to a Con, PBLC-L and PBLC-H group, which received 0, 80 and 160 mg/d of a menthol-rich PBLC, respectively. After four weeks, ruminal and jejunal epithelia were analyzed for mRNA abundances of the tight junction (TJ) genes of occludin, zonula occludens (ZO)-1 and ZO-2, and claudin (Cldn)-1, Cldn-2, Cldn-3, Cldn-4 and Cldn-7. Epithelial barrier and ion transport properties of the rumen and mid-jejunum were measured by unidirectional fluorescein flux rates, short-circuit current (Isc) and tissue conductance (Gt) in Ussing chambers. **Results:** In the rumen, PBLC pre-feeding increased mRNA expression of Cldn-7 linearly ($P = 0.01$) and ZO-2 in a quadratic manner ($P = 0.005$). In the jejunum, PBLC pre-feeding decreased both Cldn-4 ($P = 0.012$) and ZO-2 ($P = 0.006$) mRNA expression when comparing both PBLC groups with the Con group. In the rumen, Isc increased ($P < 0.001$) and Gt tended to increase ($P = 0.082$) linearly with PBLC pre-feeding, without affecting fluorescein flux rates. Absorptive fluorescein flux rates were higher than the respective secretory flux rates across all groups in the rumen only ($P < 0.001$). In the jejunum, Isc and Gt were not affected by treatment. However, absorptive fluorescein flux rates were decreased ($P = 0.006$) and secretory flux rates tended to decrease ($P = 0.095$) when comparing both PBLC groups with the Con group. **Conclusion:** Menthol-rich PBLC supplementation in the used dosages had merely minor effects on the expression of barrier forming TJ genes in rumen and jejunum that were not followed by similar changes in fluorescein flux rates. The alterations in electrophysiological properties of the rumen were also not reflected by changes in fluorescein transport. These results strongly suggest that transcellular effects of PBLC should be primarily responsible for the increased Ca^{2+} absorption in cows and sheep and that paracellular permeability of ruminal and jejunal epithelia is not measurably influenced after a four-week supplementation period with menthol-rich PBLC.

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73.

Effect of high doses of phytase on structure and physiology of large intestine epithelium in broiler chicken

Wirkung hoher Phytasedosen auf Struktur und Physiologie des Dickdarmepithels bei Broilern

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Phytic acid is the main phosphate storage compound in seeds and it is considered an anti-nutritional factor in the feed leading, for example, to a decrease in nutrient digestibility. Furthermore, it might directly or indirectly affect the intestinal mucosa and the immune system. In a previous report (1), improvements in performance, ileal P-digestibility and mineralization of the tibia were shown after phytase addition in broilers feed. The aim of this study was to determine the effects on the structure and physiology of the epithelium of the large intestine of broilers receiving increasing doses of phytase including a high dose (3000 FTU/kg feed), far beyond the average used in practice. **Methods:** For the 21-days study, 140 broiler chickens were assigned to five dietary treatments (28 birds/group). One group received a "phytate-free" diet based on Hermetia meal and corn starch (T1) while the others (T2-T5) received a "phytate-containing" diet with corn, soybean meal and sunflower meal. The "phytate-containing" diets were prepared without (T2) or with supplementation of Natuphos® E (BASF SE, Germany) at levels of 500 FTU/kg (0.01% in feed; T3), 1,000 FTU/kg (0.02% in feed; T4) and 3,000 FTU/kg (0.06% in feed; T5). The diets were formulated to meet or exceed the nutrient requirements for broiler chickens recommended by GfE (1999), and contained 12.4 MJ/kg of metabolizable energy, 224-231 g/kg of crude protein, 8.6-9.2 g/kg of calcium and 7.5 (T1) or 4.4-4.6 g/kg of phosphorus (T2-T5). Morphometry and immune-histochemistry (CD3+ intraepithelial lymphocytes, IELs) measurements were performed in caecum, using formalin fixed tissue. Gene expression of inflammatory (IFN-g, IL-1b, IL-6, IL-8) and gut barrier function (ZO-1, CLDN-5, MUC-2) markers were also analysed in mid colon tissue by real-time qPCR. Statistical analyses were performed with the software package SPSS, using one-way ANOVA with treatment as fixed effect, and followed by post hoc Tukey test. **Results:** Caecum crypt depth showed no significant differences amongst the five groups. However, when data were expressed in relation to body weight or metabolic body weight, birds receiving the diets supplemented with Natuphos® E showed lower caecum crypts depth ($p<0.001$ and $p=0.011$, respectively). This would indicate a slower renewal rate for mucosal cells registered after the administration of the phytase in comparison to the birds in T2 that would show a higher epithelial damage. Regarding CD3+ IELs, T1 and the three groups receiving the Natuphos® E showed lower counts per crypt than the birds in T2 (-47.4%, on average) and the differences also appeared, although at a slightly lower extent (-34.2%, on average), after expressing the results per 10,000 μm^2 ($P<0.001$, in both cases). Moreover, as the phytase dose increased, there was a numerical decrease in the counting of CD3+ IELs. Finally, gene expression showed significant differences for all the genes involved in immune response, after normalization with the housekeeping genes. Nevertheless, the extent of the differences was numerically low which would indicate a limited biological significance. **Conclusions:** The current results showed that the inclusion of Natuphos® E in the diet of broilers from day 1 to 21 would attenuate the immune response in the large intestine attributable to the presence of the phytic acid.

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74.

Exploring the function of the gastric barrier protein claudin-18 in a heterologous expression model

Untersuchung der Funktion des Magenbarriere-Proteins Claudin-18 in einem heterologen Expressionsmodell

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Claudins are integral membrane proteins of Tight Junctions (TJ), which determine the permeability of the paracellular pathway and therefore organ-specific barrier properties of epithelia. The claudin-18 type 2 splicing form is the predominant claudin in gastric epithelium. It is regarded to protect the submucosa from protons of the gastric lumen by forming tightly anastomosing TJ strands, that are related to the function of a proton barrier [1]. The deficiency of claudin-18 leads to dysregulation of the paracellular pathway as well as H^+leakage, and causes chronic gastritis and gastric tumorigenesis [2]. Recently, we established Xenopus laevis oocytes as a heterologous expression system for TJ proteins [3]. In our current study, we analyzed pH-dependency of claudin-18 homophilic trans-interaction in this model.
Methods: Human claudin-18-2 cDNA was cloned into a suitable pGEM-HE_MCS vector for *in vitro* transcription. Subsequently, 1 ng claudin-18 cRNA was injected into Xenopus laevis oocytes. Oocytes injected with RNA-free water served as negative controls. After 3 d, claudin-18 expression was detected using immunoblot and immunofluorescence microscopy. Pig jejunum served as a positive control. For analysis of tight junction integrity, the vitelline membranes of claudin-18 expressing oocytes were removed, and cells were clustered in combinations of claudin-18 + claudin-18, claudin-18 + control, and control + control. Culture medium was ranging from pH 5.5, 6.5 to 7.5. Next, contact width of the oocyte pairs were measured after 1 h and 24 h. To evaluate the data, the contact areas were calculated with the formula of circle equation. For statistical analysis, normal distribution was checked using Shapiro-Wilk test and controls were set as 100%. One-way ANOVA was used for multiple comparison of normalized data and Dunnett's test for the correction of multiple testing. Kruskal-Wallis test was performed for non parametric data.
Results: Immunoblots of claudin-18-injected oocytes detected specific bands at 27 kDa in accordance with the predicted size. In water-injected oocytes no signal was detected. Moreover, immunofluorescence microscopy confirmed a localization of claudin-18 in the membrane of cRNA-injected oocytes. The paired oocyte incubation experiment revealed no significant change of contact areas of claudin-18-expressing cells after 24 h at pH 7.5: 18 + ctrl: 116,3 ± 13,5 %; 18 + 18: 122,9 ± 29 % (one-way ANOVA: $F(2, 13) = 0{,}18$; $p = 0{,}84$; $n = 3\text{-}7$) and pH 6.5: 18 + ctrl: 105,7 ± 23,2 %; 18 + 18: 80,6 ± 24,8 % (Kruskal-Wallis test: $H(2) = 0{,}21$; $p = 0{,}9$; $n = 2\text{-}7$), whereas the combination of claudin-18-expressing oocytes at pH 5.5 showed a reduced contact surface of 67 % compared to controls: 18 + 18: 66,7 ± 6,8 % ($p = 0{,}023$; one-way ANOVA: $F(2, 18) = 4{,}08$; $p = 0{,}035$; $n = 5\text{-}10$).
Conclusion: The incubation experiment enables an analysis of the homologous interaction of claudin-18 depending on pH value in order to obtain conclusions about the strength of the anastomosis of the TJ-like strands formed by claudin-18. As contact areas of claudin-18 expressing oocytes at pH 5.5 are reduced, more interaction experiment will be carried out to characterize the pH dependency of the trans-interaction of claudin-18 in more detail.

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75.

TNFα induces changes in the composition of tight junction proteins in follicle-associated epithelium of porcine Peyer's Patches

TNFα führt zu Veränderungen in der Zusammensetzung von Tight Junction-Proteinen im follikel-assoziierten Epithel von porzinen Peyerschen Platten

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Peyer's patches (PP), a major part of the gut-associated lymphatic tissue, are located in the distal small intestine and are covered by the follicle-associated epithelium (FAE), which controls the interaction between antigens from the gut lumen and the immune system (1). Compared to the surrounding jejunal villus epithelium (VE), PP shows a stronger epithelial barrier function (2). Recent studies showed that Tumor necrosis factor alpha (TNFα), a proinflammatory cytokine, which is involved in various local and systemic diseases, is able to alter tight junction proteins and therefore affects the intestinal epithelial barrier function (3). Our study aimed to identify whether TNFα has an effect on porcine Peyer's patches, compared to surrounding villus epithelium, employing the Ussing chamber technique.

Methods: Epithelial tissue samples with and without PP from the distal jejunum of adult pigs were mounted into Ussing chambers. For examining the effects of TNFα, the cytokine was added in different concentrations (5,000 and 10,000 U/mL) to the basolateral side of the tissue. During the experiment, the transepithelial electrical resistance (TEER), representing the epithelial barrier function, was measured for at least 10 hours to evaluate the effect of the cytokine in the different tissues. After 3, 6, and 8 hours, a buffer exchange was carried out to ensure the viability of the tissue. Subsequently, tissue samples were further processed for immunoblotting, using specific antibodies raised against tight junction proteins. Statistical analysis was performed using one-way ANOVA for TEER measurements, and Student's t-test for analysis of Western Blot bands. P-values below 0.05 were considered to be statistically significant.

Results: After nine hours in the Ussing chamber, 5,000 U/mL TNFα showed a tendency towards a decrease of TEER in PP tissue compared to the untreated controls (set as 100%): 0 U/mL: 97.8 ± 4.0 %; 5,000 U/mL: 86.1 ± 3.2 %; 10,000 U/mL: 89.9 ± 3.2 % (one-way ANOVA: $F(2, 39) = 2.88$, $p = 0.068$, $n = 14$). Conversely, no trend towards changes in TEER could be observed in VE during the experiment: 0 U/mL: 126.9 ± 15.8 %; 5,000 U/mL: 113.0 ± 11.9 %; 10,000 U/mL: 108.2 ± 13.7 % (one-way ANOVA: $F(2, 39) = 0.49$, $p = 0.62$, $n = 14$). In PP, immunoblotting of protein samples from selfsame tissues indicated significant changes in total expression of single members of the claudin tight junction protein family, namely claudin-1 (52.4 ± 13.3 %, $p = 0.012$, $n = 5$), and claudin-4 (55.8 ± 5.8 %, $p = 0.0003$, $n = 4$). Additionally, a marked increase of the pore-forming TJ protein claudin-2 could be observed in PP (149.3 ± 16.8 %, $p = 0{,}012$, $n = 4$). None of the above mentioned claudins showed changes in VE after TNFα-treatment.

Conclusion: Our current study demonstrates not only the basic differences of epithelial barrier function between PP and VE. For the first time, also a different regulation was observed, as TNFα only had a significant effect on the total expression of various TJ proteins in porcine PP while surrounding VE remained unchanged. Thus, the importance of this structure as a primary inductive site of mucosal immunity was verified. Due to the tendency towards significance of changes in TEER in PP, further experiments need to be performed, to further explore the effects of the cytokine on epithelial barrier function.

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76.

Novel insights in metabolic status of Holstein fattening bulls

Neue Erkenntnisse zum Stoffwechselstatus von Holstein Mastbullen

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Intensive fattening based on high amounts of easy digestible and starch-rich concentrate is a common practice to fatten bulls. However, chronic excessive dietary energy intake is known to put animals to risk of developing metabolic disorders. In humans, these are commonly labelled as obesity-related morbidities such as the human metabolic syndrome. In companion animals, similar concepts have been established for horses, i.e. the equine metabolic syndrome in relation to a carbohydrate-rich diet and sedentary lifestyle. In fattening bulls, intensive feeding is traditionally accepted as a method to achieve production targets; however, excessive intake of carbohydrate-rich diet is also known to cause rumen acidosis, laminitis or tail tip necrosis, commonly referred to as production diseases. Metabolic status of that condition is not described in detail. By the technique of metabolomics, a snapshot of the concentrations of a wide array of metabolites in a biological sample can be obtained. As these metabolites are intermediary or end products of various metabolic pathways, this provides a good indication of the current metabolic status. Therefore, the aim of this study was to elucidate changes in metabolite profiles of intensively fed Holstein bulls and to identify novel metabolites and affected pathways, which are associated with laminitis. **Methods:** Thirty Holstein bulls intended for beef production were randomly assigned to an intensive (IN) (n = 15) or a moderate (MO) nutritional regimen (n = 15). Diets were based on corn- and grass-silage and IN received a surplus of 6 kg concentrate/day/animal for the last 7 months of the fattening period. Bulls were weighed monthly. Average daily gain (ADG) was calculated from body weight with the exact number of days between weighing. Clinical signs of laminitis were documented and blood plasma samples were collected at the beginning and end of experimental period (slaughter). Bulls were slaughtered at the age of 20 months. Insulin concentrations (ELISA; Mercodia AB, Uppsala, Sweden) in serum and the metabolite profiles (AbsoluteIDQ® p180 kit, Biocrates, Innsbruck, Austria) in plasma were analyzed. Results between feeding groups were compared by unpaired student's t-test. Level of significance was set at $P < 0.05$. **Results:** ADG of IN during feeding trial was 1474 g, of MO 957 g ($P < 0.0001$). All IN bulls showed signs of chronic laminitis, but none of MO at slaughter. Metabolite profiles revealed complex alterations of various metabolic pathways. 90 of 182 analyzed metabolites were significantly different between feeding groups. Compared to MO bulls, lower concentrations were found in the substance classes of phosphatidylcholines (PC) and sphingomyelins in IN bulls. In contrast, the concentrations of lyso-PC and amino acids were higher in IN bulls. Within the amino acids, the branched-chain amino acids (BCAA) were particularly different (isoleucine ($P < 0.001$), leucine ($P < 0.001$), valine ($P < 0.001$)). Concentration of insulin in the plasma was also higher ($P < 0.0001$) in intensively fed bulls. **Conclusion:** Strong differences in plasma metabolite profiles were observed, due to intensive fattening. Metabolic status of intensively fed bulls was associated with the metabolic disorder laminitis. Hyperinsulinemia and enhanced BCAA are characteristic features. Future research is warranted to understand (patho-) physiological pathways of laminitis in cattle.

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77.

Milk MIR-spectra based estimates for evaluation of dairy cow health at a cow-individual and at a herd level

Milch MIR-Spektren basierte Schätzwerte zur Beurteilung der Milchkuhgesundheit auf Einzeltier- und Herdenebene

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The early lactation period is accompanied by an enhanced lipolysis in adipose tissue, which results in greater concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) in blood. Besides the decline in feed intake and milk production, subclinical ketosis (SCK) can trigger the development of further health issues associated with reduced animal performance such as clinical ketosis (CK). The primary aim of this study was to evaluate the suitability of MIR spectra-predicted acetone and BHB in milk for early detection of elevated blood BHB concentrations in individual cows or at a herd level. **Methods:** In experiment 1, blood and milk samples were taken every two weeks after calving up to d 125 and once around d 200 from Holstein (n=80), Brown Swiss (n=72) and Swiss Fleckvieh (n=58) cows. In experiment 2, cows diagnosed with CK (n=474) and 420 control samples with blood β-hydroxybutyrate [BHB] < 1.0 mmol/L were used to investigate if CK could be detected by FTIR-predicted BHB and acetone from a preceding milk control. In experiment 3, correlations between data from an in farm automatic milk analyzer (DeLaval Herd Navigator, HN) and FTIR-predicted BHB and acetone from the monthly milk controls were evaluated. Blood samples were analyzed for BHB, and acetone was measured in milk. MIR-spectrum based measurements of milk fat and protein, as well as prediction of BHB and acetone concentrations in milk were done on a MilkoScan FT 6000 analyzer using the corresponding prediction models from FOSS (Foss, Hilleroed, Denmark). We used Pearson's correlation coefficients to investigate the relationship between blood BHB concentration, acetone concentration in milk, and the MIR spectra-predicted milk BHB and acetone concentrations. The repeated measures mixed model analysis (SAS, version 9.4) was performed to examine the effects of breed, farm and parity number. The individual cow was considered as repeated subject. The Tukey-Kramer post hoc test was used for detection of significances at $P<0.05$. Contrasting of weekly summarized milk BHB concentrations predicted from DHI samples in CK cows against appropriate controls without CK was performed by paired t-tests. **Results:** Only 3.6% out of the samples obtained biweekly in exp. 1 had blood BHB concentrations ≥ 1.0 mmol/L. Samples with blood BHB concentrations < 1.0 mmol/L were considered non-SCK. There was no effect of breed on blood BHB concentrations ($P=0.48$) and milk acetone measured chemically ($P=0.28$). The correlation between blood BHB and milk MIR spectra-predicted BHB was low ($r=0.37$). In terms of MIR spectra-predicted acetone in milk, the overall correlation with blood BHB concentration was low ($r=0.12$). Holstein cows in exp. 2 that were diagnosed with CK (n=66) had greater MIR spectra-predicted BHB and acetone concentrations in milk (0.15 ± 0.03 mmol/L and 0.22 ± 0.04 mmol/L, resp.) compared with non-ketotic Holstein cows of similar performance and lactational stage (-0.01 ± 0.00 mmol/L and -0.03 ± 0.00 mmol/L; $P<0.05$). Similar results were found in Brown Swiss cows identified with CK (n=408). On the day of CK diagnosis, we observed a high variation of estimated ketone body concentrations in milk. The analysis of individual milk BHB profiles based on HN measurements (exp. 3) indicated that 15.6% of all cows had at least one milk BHB concentration greater 0.12 mmol/L. A moderate positive correlation was found between the direct enzymatic milk BHB measurements by the HN and the indirect MIR spectra-predicted milk BHB content obtained from concomitantly DHI recordings on the same day ($r=0.61$; $P<0.0001$). Assuming the HN alarms at milk BHB concentrations of 0.12 mmol/L as reference criterion, approximately 90% of the hyperketonemia cases would have been missed by the official milk control performed approximately only once per month. **Conclusion:** The predictive value of MIR spectra-predicted concentrations of ketone bodies in milk is limited in terms of an early detection of SCK when dairy cows are experiencing a moderate metabolic load. The indirect assessment of milk ketones by FTIR spectrometry does not reliably detect hyperketonemia in individual samples, but is more reliable if blood BHB concentrations are above the thresholds of SCK and CK diagnosis.

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78.

Influence of a menthol-rich feed additive on calcium homeostasis and performance in peripartal dairy cows

Einfluss eines Menthol-haltigen Futterzusatzes auf Kalzium-Homöostase und Leistung bei peripartalen Milchkühen

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Calcium homeostasis is a serious problem during the peripartal period in dairy cows. A sharp drop in blood calcium levels leads to clinical manifestation in downer cows; however, a less severe drop into the subclinical range already increases the risk of secondary diseases. The prevalence of subclinical hypocalcemia in multiparous dairy cows is very high, up to 60% [1]. Recent studies have shown that the administration of plant bioactive lipid components (PBLC) like menthol can increase the absorption of calcium in the rumen [2-3]. This effect is most likely based on a stimulation of epithelial cation channels of the transient receptor potential (TRP) family [3]. The hypothesis of the present feeding study was that menthol exerts positive effects on calcium homeostasis in Holstein Friesian (HF) and Brown Swiss (BS) cows with possible outreach on performance parameters.

Methods: The experiment included 84 multiparous cows (47 HF and 37 BS) on a commercial farm with no prophylaxis against hypocalcemia. The cows in each breed were allocated into one control (CON) and one PBLC-treatment (TRT) group according to lactation number, previous 305-d milk yield and calving date. The trial began 8 d before the expected calving date and ended at 80 d postpartum. During this time, the cows in TRT were fed 1.2 g of a mixture of PBLC (catiBovin, PerformaNat GmbH, Germany, with >80% menthol as main ingredient). Before calving the additive was fed as an on top solution, after calving the additive was fed via a dosing unit of the milking robot. On days -2, 1, 3, 5, 7, 14, 21 and 28, a blood sample was taken from the coccygeal vein and ionized calcium (iCa), potassium, sodium, chloride and glucose were determined. Daily milk yield was recorded by a milking robot and the milk composition was analysed in two test periods (4 to 40 d and 41 to 72 d). The incidences of diseases were also determined. The latter included clinical hypocalcemia (iCa < 0.8 mmol·l-1 and recumbent), subclinical hypocalcemia (iCa < 1.0 mmol·l-1), and metritis. Measurement of feed intake was not possible. Statistical evaluation was performed using a linear mixed model with the MIXED procedure of SPSS 26. Significance was assumed at $P < 0.05$.

Results: Feeding PBLC resulted in elevated blood calcium levels in HF ($P < 0.05$), but not in BS. Interestingly, the iCa of TRT HF was higher than that of TRT BS ($P < 0.05$). Blood concentrations of sodium, potassium, chloride and glucose were not affected by treatment. HF cows produced more milk than BS cows ($P < 0.001$), but milk yield was increased by treatment of both breeds ($P < 0.01$). The milk composition (g/kg) was not changed by treatment, however, there was an increased production of fat, protein and lactose in kg/d in treated cows across breeds ($P < 0.05$). No differences were found in milk somatic cell count and urea levels. The incidence of subclinical hypocalcemia was 23.8% in CON and 14.7% in TRT. Clinical milk fever could be detected only in the HF group (CON = 8% vs. TRT = 5.6%). The incidence of metritis was 40.7% in CON and 14.7% in TRT.

Conclusion: Supplementing a menthol-rich PBLC in the periparturient period improved calcium homeostasis of the HF breed as indicated by increased iCa in the blood of TRT HF cows. A similar effect could not be observed in TRT BS cows, which may be linked to the fact that this breed is much more robust against hypocalcemia. Supplementation of PBLC further reduced the incidence of metritis and increased milk yield in HF and BS breeds with no effects on milk composition. The improved metritis incidence and milk yield may suggest that the supplied PBLC may have effects beyond increased gastrointestinal calcium absorption via TRP channels, e.g., on metabolism and the immune system.

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79.

P-Fowl: Does the onset of egg-laying differently affect the myo-inositol metabolism and metabolite profiles of brown and white hens?

P-Fowl: Beeinflusst das Einsetzen der Eiablage den Metabolismus und die Profile von Myo-Inositol im Plasma von braunen und weißen Hennen unterschiedlich?

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Egg laying onset is a challenging metabolic period during which hens go through several physiological adaptations such as medullary bone formation and final reproductive tract maturation [1]. This research aimed to assess the effects of egg laying onset and hen strain on the blood metabolite profile, generating better understanding of underlying metabolic adaptations. **Methods:** 20 Lohmann-Classic Brown (LB) and 20 Lohmann LSL-Classic (LSL) hens were kept in metabolism units at two stages of production (16 and 24 weeks of age). All hens were provided the same nutrient-adequate diet specific for each age [2]. Liver, muscle and kidney samples were analyzed spectrophotometrically to quantify myo-inositol (MI) concentrations and key MI enzyme expression (MI monophosphatase (IMPase 1) and myo-inositol oxygenase (MIOX)) whereas metabolites from trunk blood were identified by the targeted AbsoluteIDQ p180 Kit from Biocrates [3]. Two Way Anova was used to compare MI metabolism, Principal Component Analysis (PCA) was performed for metabolite profiling, and Venn diagrams from student`s t-test results were performed to identify metabolites differing between age and strains. Statistical difference was set as false discovery ratio (FDR) adjusted P value less than 0.05. **Results:** With regards to MI metabolism, only MIOX expression was different between periods and strains (FDR-adjusted <0.05). PCA demonstrated marked differences in metabolite profiles at week 24 among individuals in both strains in comparison to week 16, but no clear difference between strains. Venn diagram showed that 17 metabolites changed equally in both strains at week 24 (9 amino acids, 5 biogenic amines, sum of phospholipids and sum of hexoses). Only lysine changed in LB hens whereas 15 metabolites, mainly consisting of amino acids (7) and biogenic amines (6) and sum of hexoses changed exclusively in LSL hens (FDR-adjusted $p<0.05$). **Conclusion:** LB and LSL might have distinct MI regulation and metabolite plasma profiles during the onset of egg laying. These findings indicated a differential metabolic profile associated to energy, amino acid and lipid metabolism, which might involve processes of protein turnover, energy regulation, oxidative stress, and inflammation pathways. Present findings may stimulate further research on nutritional interventions pointing to specific metabolic needs; however, more research is necessary to elucidate metabolite interactions during onset of laying.

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80.

Insulin and Mg^{2+} influence the expression of Mg transporters and of genes with central metabolic functions in the bovine liver cell line BFH12

Insulin und Mg^{2+} beeinflussen die Expression vom Mg Transportern und Genen mit zentralen metabolischen Funktionen in der bovinen Leberzelllinie BFH12

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During the transition period of approximately three weeks before and after calving, the energy demand of dairy cows increases tremendously. This phase is also characterized by a reduced insulin sensitivity in, e.g., skeletal muscles and adipose tissue [1]. In this phase of glucose shortage, ketone bodies serve as an energy source for peripheral tissues. They are synthesized in the liver from fatty acids mobilized from adipose tissue. Excessive lipid accumulation negatively affects the physiology of the hepatocytes and may result in fatty liver and ketosis. Mg^{2+} and insulin have eminent influence on the metabolism in hepatocytes. Not only that Mg^{2+} is indispensable for the efficacy of insulin, it also influences the activity of many enzymes that require the ion as co-factor. The understanding of the relationship between magnesium and insulin in metabolic processes of the liver shall provide the basis for new therapeutic strategies of many metabolic diseases of dairy cows.

Methods: In the present study, the bovine hepatocyte cell line BFH12 [2] was used as *in vitro* model and the effect of various Mg^{2+} and insulin concentrations on the expression of genes involved in Mg homeostasis as well as of genes with central functions in liver metabolism were investigated. Cells were cultured in a modified Williams E Medium with a reduced amount of glucose (3.3 mmol/L) and a physiological insulin concentration (100 pM). The medium was furthermore supplemented with β-hydroxybutyrate, acetate, and propionate [2]. Proceeding from this basal medium, cells were exposed for 24 hours to various Mg (0.2; 0.8; 1.2 and 1.5 mM) and insulin (100 pM, 500 pM und 50 nM) concentrations. After exposure to these different growth conditions, the expression of genes involved in Mg homeostasis and gluconeogenesis were analyzed by quantitative RT-PCR in triplicates. Relative expression was determined by the ΔΔCt method (reference genes beta-actin, RPS19 and YWHAZ) and analyzed by two way analysis of variance (ANOVA).

Results: Among the genes with involvement in Mg homeostasis, TRPM6 and SLC41A1 exhibited the most prominent changes in expression. For TRPM6 a statistically significant interaction between the factors magnesium and insulin ($P < 0.001$) was identified. Expression of the gene increased with increasing Mg concentrations and concomitant high insulin availability ($P < 0.001$). On the contrary, a very low Mg-concentration (0.2 mM) resulted in the decreased expression of TRPM6 independent of the insulin concentration used ($P = 0.002$). Expression of SLC41A1 in hepatocytes was mainly influenced by insulin ($P = 0.003$) with high insulin concentrations stimulating the expression of the gene. Varying effects of insulin were identified for several genes involved in lipid metabolism (ACCA, ACSL, CPT IA, PCCB; $P < 0.001$). Among the genes of the gluconeogenic pathway, the strongest response was observed for PEPCK1, which is a rate-limiting enzyme of gluconeogenesis and requires Mg^{2+} as cofactor [3]. An interaction between the factors magnesium and insulin ($P = 0.017$) was observed for the expression of this gene. Magnesium caused a dose-dependent increase ($P = 0.025$) in expression when insulin was present at high concentrations ($P < 0.026$). Low Mg^{2+} availability resulted in low expression and under these conditions, insulin had no influence on the expression level of PEPCK1.

Conclusion: Our results demonstrate that the expression of genes involved in Mg homeostasis is influenced by the availability of Mg^{2+} and insulin in the growth medium. SLC41A1, the most important Mg^{2+} efflux system, was among the genes that showed the strongest response to the different culturing conditions. Furthermore, our study gives a first indication that gluconeogenesis might be less efficient under conditions of low Mg^{2+} availability with simultaneously reduced insulin sensitivity, a situation that is often found in animals with excessive lipid mobilization in the transition period.

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81.

Integrated serum proteome profiles, metabolites, and hormones and their metabolite–protein networks: association with energy metabolism and the somatotropic axis in transition dairy cows

Integrierte Serumproteomprofile, Metabolite und Hormone und deren Metabolit-Protein-Netzwerke: Zusammenhänge mit dem Energiestoffwechsel und der somatotropen Achse bei Milchkühen um den Zeitraum der Abkalbung

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The integration of large omics datasets with classical blood profiling variables in dairy cow studies may provide complementary insights into the complex pathophysiology of the adaptation to the metabolic needs of lactation. The rapid increase in milk production after calving challenges the lipid and glucose metabolism and is accompanied by adaptative responses in regulatory systems such as the somatotropic axis. Identification of proteins involved in the regulation of metabolic adaptation may be of relevance in the context of postpartum energy balance (EB) and the incidence of metabolic disorders. Herein, we aimed to determine associations between individual proteins out of the serum proteome with "classical" metabolites and hormones for creating new hypotheses on the molecular drivers of the metabolic adaptation to lactation.

Methods: From sixteen Holstein cows in their second lactation, the plasma concentrations of non-esterified fatty acid (NEFA), insulin, beta-hydroxybutyrate (BHB), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), as well as growth hormone and insulin-like growth factor (IGF)-I, were measured on days -21, 1, 28, and 63 relative to calving (1, 2) and related to the proteome characterized at the same time-points using nano-scaled liquid chromatography coupled with tandem mass spectrometry. Integration analysis of plasma metabolites and hormones, and serum proteome profile (comprising 241 proteins) was performed using sparse partial least squares (sPLS) regression model in the mixOmics package (http://mixomics.org/, R statistical software) and the top covariates were validated by Pearson's r correlation analysis (corrplot package, R statistical software) with $P<0.05$. Identified proteins were mined for functional enrichment analysis and protein-protein interaction using the ProteINSIDE (https://proteinside.org/).

Results: IGF-I, NEFA, and LDL-C were identified as the top correlated classical variables with serum proteins. The sPLS model revealed a correlation between IGF-I and IGF-binding protein (IGFBP2) (r= -0.71), and a moderate correlation between LDL-C, Apolipoprotein B (APOB) (r=0.53), IGFBP2 (r= 0.5), and Prenylcysteine Oxidase 1 (PCYOX1) (r= 0.5). There was also a correlation between NEFA and IGFBP2 (r=0.59), and APOB (r= -0.5), IGFBP Acid Labile Subunit (IGFALS) (r= -0.5), and Alpha 2-Heremans-Schmid Glycoprotein (AHSG, r= -0.47). Pathway enrichment analysis revealed that ApoB, IGFALS, IGFBP2, and AHSG were interconnected and are involved in the regulation of IGF transport and uptake by IGFBPs. For PCYOX1, bioinformatics analysis in the Homo sapiens database indicates an interaction with APOB in LDL metabolism. Moreover, all the proteins established herein with correlations are associated with glucose metabolism, the somatotropic axis, and insulin resistance.

Conclusions: Integration analysis combined with bioinformatics revealed associations between circulating proteins from proteomics with metabolites and hormones and identified common biological functions related to the regulation of lipid and glucose metabolism and insulin resistance. The identified proteins, in particular IGFALS, PCYOX1, and AHSG that were not considered in transition cow biology until now, might point to further pathways that are relevant in this context.

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82.

Associations of the peripartal loss of back fat thickness (BFT) and the ante-partum BFT with selected blood variables in transition dairy cows: A cluster analytic approach

Beziehung zwischen dem peripartalen Verlust an Rückenfettdicke (RFD) sowie der RFD ante partum und verschiedenen Blutparametern bei Milchkühen in der Transitphase: ein Cluster-analytischer Ansatz

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The transition from late pregnancy to early lactation in dairy cows requires comprehensive metabolic adaptations to the changing nutritional status. The extent of body fat mobilization during this transition period varies between cows and usually shows a positive correlation with the ante partum (ap) body condition of the animal. For investigating the relation between body fat and metabolic adaptations during the transition period, cows are usually classified by using arbitrarily defined thresholds for body condition or BFT. However, this is not necessarily the best possible grouping and is inefficient for large data sets. Our objective was thus (a) to generate clusters of cows during the transition period using data of their BFT by an unsupervised machine learning algorithm, and (b) to compare the clusters in terms of the serum concentrations of selected blood variables established as indicators of metabolic and inflammatory status.

Methods: We included data from BFT (mm; ultrasound scan, in the sacral region), and serum samples collected 25 ± 10 days ap and 30 ± 3 days (means ± SD) post partum (pp) from 713 cows in one dairy farm. Cows were subjected to cluster analysis as follows: In Approach 1, the delta BFT (ΔBFT) was calculated by subtracting the BFT-pp from the BFT-ap; unsupervised clustering analysis (k-means) was performed to construct clusters based on ΔBFT. Five clusters were identified: extreme loss (EL, ΔBFT from 17 to 23 mm, n = 16), moderate loss (ML, 9 to 15 mm, n = 119), small loss (SL, 4 to 8 mm, n = 326), no loss (NL, 0 to 3 mm, n = 203), and gain (GN, -8 to -1 mm, n = 51). In Approach 2, the k-means clustering was based exclusively on the BFT ap. Five clusters were identified: lean (LEAN, BFT from 5 to 8 mm, n = 50), normal (NOR, 9 to 12 mm, n = 206), slightly fat (SF, 13 to 16 mm, n = 202), just fat (JF, 16 to 22 mm, n = 194), and very fat (VF 23 to 43 mm, n = 61). Beta-hydroxybutyrate (BHB) and fatty acids (FA), and two metabolic hormones (leptin and adiponectin) were measured both ap and pp. The data from the two trials were then analysed in SAS using the PROC MIXED (SAS Institute Inc., Cary, NC) with repeated measures and with cluster and parity as fixed effects, and cow set as random effect. The threshold of significance was set at $P \leq 0.05$; trends were declared at $0.05 < P \leq 0.10$.

Results: When considering Approach 1 (ΔBFT), the pp serum concentrations of BHB and FA were greater in ML than in SL ($P = 0.01$ and $P < 0.01$), NL ($P < 0.01$ and $P < 0.01$), and GN ($P < 0.01$ and $P < 0.01$). The serum concentration of adiponectin ap was higher in ML compared to EL ($P = 0.03$). Cows gaining BFT (7% of the studied animals) had greater concentrations of adiponectin ap than EL animals ($P = 0.02$). In Approach 2 (BFT ap), the ap serum concentration of leptin tended to be higher in VF than in LEAN ($P = 0.06$) and there was a trend to greater values in JF when compared to LEAN ($P = 0.08$). The FA serum concentration pp was greater in VF compared to LEAN ($P = 0.05$) and tended to be higher in VF compared to NOR ($P = 0.08$). Moreover, 98% of the VF cows, 88% of the JF cows, and 69% of the SF cows were also classified in the clusters loosing BFT (EL, ML, and SL), whereas 57% and 100% of the NOR and LEAN cows, respectively, were not losing or even gaining BFT.

Conclusions: Clustering analyses yielded five clusters of animals in both approaches with distinct differences in BFT loss and in BFT ap, respectively. Considering the variables assessed in blood, our results confirm that cows with greater losses of BFT also have elevated lipolysis, Cows gaining BFT were not different from normal cows regarding their serum variables. Moreover, higher levels of leptin were associated with fatter animals, whilst greater concentrations of adiponectin ap were observed in cows with moderate BFT loss and in cows gaining BFT regardless of their BFT ap. This may indicate greater insulin sensitivity promoting adipogenesis and inhibiting lipolysis. Overall, our data confirm that cows with greater BFT are more prone to lose BFT when entering lactation.

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83.

Retrospective field study on risk factors of tail biting in pigs

Retrospektive Feldstudie zu den Risikofaktoren der Caudophagie bei Schweinen

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Tail biting is a common problem in pig farming. Pigs affected by tail biting suffer from serious pain, injury and diseases. To prevent tail biting tail docking in piglets has become a routine procedure used by piglet producers in Germany. This procedure is not in accordance with EU legislation. Hence, it is necessary to find effective strategies to resolve the problem of tail biting amongst pigs without the need of tail docking. The working hypothesis was to find correlations between gastrointestinal health and tail lesions originating from tail biting with a focus on the nutritional history.

Methods: Based on data collected at a German abattoir, 20 commercial fattening pig farms were selected to participate in this study. Depending on pathological findings which all were classified as results of tail biting the participating fattening pig farms were assigned to a high risk and a low risk farms. Ten low risk farms (LRF) without history of tail biting for the previous 18 months were compared to 10 farms with pathological findings observed in 1 – 5 % of the slaughtered pigs (high risk farms, HRF). All participating farms were evaluated using a questionnaire which focused on nutrition, feed composition and management. In nine LRF and ten HRF, feed was sampled for further analysis (particle size, Weende analysis). Post mortem analyses of the carcasses and the gastrointestinal tract of the slaughtered pigs from participating farms were performed. Ten healthy pigs from LRF, which showed no tail lesions and 30 pigs from HRF, which were assigned to three different groups based on condition of their tails (10 pigs with no tail lesions, 10 pigs with fresh acute tail wounds and 10 pigs with chronic healed tail wounds). The gastrointestinal tract was scored visually and histologically, in addition TLR-5, IL-8, IL-10, IL-17 and the natural killer cell receptor NKG2D were measured by real-time quantitative PCR. The serum concentration of the acute phase protein haptoglobin was measured as an indicator of inflammatory processes. The statistical analysis was performed by SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA), depending on data distribution by t-test or Mann-Whitney U-test ($p<0.05$).

Results: Differences in particle size or in nutrient concentrations of the diets could not be detected between HRF and LRF. Only the crude fibre content in the feed from the HRF tended to be lower compared to the LRF ($p = 0.065$). All the farms offered their pigs various enrichment materials such as chains, plastic elements, pieces of wood, movable plastic pipes, strings made of sisal fibers as well as playing balls. The legal requirement for drinkers was fulfilled in all farms according to teh current regulations. In HRF pigs 87 % of animals showed alterations of the gastric mucosa, in 30 % gastric ulcers could be detected. In comparison, 50 % of the LRF pigs showed alterations in the gastric mucosa, only one animal with gastric ulcer. The expression rate of TLR-5, NKG2D and IL-8 increased in HRF pigs with acute lesions ($p <0.05$), IL-17 and IL-10 showed no differences in animals from LRF and HRF. HRF pigs with chronic tail lesions had a reduced expression of NKG2D ($p<0.05$). Histological inflammation scores of the small intestine and colon showed the highest rate of inflammation in pigs with acute and chronic lesions from HRF when compared to the pigs from LRF ($p<0.05$). The serum haptoglobin level was highly variable, there were no statistically significant differences between the groups ($p=0.577$).

Conclusion: The results indicate inflammatory processes in the gastrointestinal tract of pigs with acute and chronic tail lesions from HRF farms. For further hypothesis building, studies on potential mechanisms will be needed.

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84.

Expression profile of chosen candidate genes regulating the adaptive immune system within ileal tissue of pigs after feeding transglycosylated starch

Expressionsprofil von ausgewählten Kandidatengenen des adaptiven Immunsystems in ilealen Gewebe von Schweinen nach einer Fütterung mit transglykolisierter Stärke

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Transglycosylated starch (TGS) type 4 is a chemically modified starch which promotes health by modulating the gastrointestinal microbiota, the lipid metabolism, and genes of the innate immune system as well as reducing blood glucose and insulin in the immediate postprandial phase (1). The aim of this study was to assess the impact of transglycosylated starch on genes of the adaptive immune system regarding its proposed anti-inflammatory effects. Previously, we could demonstrate that TGS affects the expression of genes of the innate immune system (1); little evidence exists yet on its impact on the adaptive immune system. Therefore, we selected important driver genes of the adaptive immune response and evaluated their expression level in the ileal mucosa of growing pigs.

Methods: A total of 16 barrows were divided in two groups, either fed the control diet or the TGS diet for 11 days. Relative gene expression of important genes within the regulation of the adaptive immune system was analyzed after isolating RNA from ileal mucosa scrapings using real-time PCR. Type 2 T-helper cell response was assessed by analysing the expression of GATA Binding Protein 3, interleukin 4 and interleukin 5. Type 1 T-helper cell response was evaluated by the expression of T-box transcription factor TBX21. For type 17 T-helper cell response the expression of RAR-related orphan receptor gamma (RORγt), interleukin 17 and interleukin 23 was quantified and for regulatory T-cells the expression of forkhead box P3 (FOXP3) was assessed. To analyse the cytotoxic T-cell response, interleukin 2 and interleukin 12 were measured. CD40 and CD2 were quantified to evaluate the response of B-cells.

Results: The interleukins 4 and 5, which are associated with a more chronic inflammatory Th2 response, showed a reduced expression in pigs fed the TGS compared to the control diet ($p < 0.05$). The interleukin 23A gene which is associated with a highly pro-inflammatory Th17 cell response showed also a decreased relative expression level of 0.46 ($p < 0.005$) within pigs fed the TGS diet compared to the control. Additionally, we detected a lower expression level 0.89 of the TBX21 gene, the master transcription factor for pro-inflammatory Th1 cells, in ileal tissue of TGS fed pigs compared to the control ($p < 0.05$). Expression of genes associated with an inflammatory T-helper cell response were downregulated in pigs fed the TGS diet compared to the control diet, whereas FoxP3, the master transcription factor for regulatory T helper cells, showed a tendency towards a slight upregulation (1.31) when feeding the TGS diet ($p < 0.1$). Chosen genes associated with a cytotoxic T cell response did not show a regulation by diet, but a broad range between the samples. For analyzing the B-cell response CD2 and CD40 have been measured. The gene expression of CD40 decreased ($p < 0.05$) with the TGS diet.

Conclusion: The results of the study indicate a regulation of five genes of the adaptive immune system after feeding the TGS diet. The expression of IL-23A, TBET, IL-4, IL-5, and CD40 was decreased, possibly leading to reduced sites of inflammation while the expression of the gene FoxP3 associated with regulatory T-cell response was slightly increased with the TGS diet. This study suggests a role of TGS in reducing inflammation in the ileum. As the adaptive immune system consists of an immunology memory also long term effects on a balanced health status after consuming a TGS diet could be possible. An effect of TGS on both the innate and adaptive immune response is important for animal as well as human nutrition and implicates treatment possibilities concerning inflammatory diseases. However, to obtain more robust results, it is highly recommended to conduct further studies.

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86.

Establishment of an *in vitro* cell culture system with avian PBMCs to assess the immunomodulatory effects of feed additives

Etablierung eines in vitro Zellkulturmodells mit aviären PBMCs für die Untersuchung immunmodulatorischer Effekte von Futtermittelzusatzstoffen

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The isolation of pure peripheral blood mononuclear cells (PBMCs) is complex and difficult in the avian species since they contain a significant number of nucleated thrombocytes and erythrocytes using common isolation methods. *Ex vivo* analyses of adaptive immune cell responses to infections and vaccination (1) and other stimuli as feed additives require efficient and pure isolation of PBMCs. The functional analysis of feed additives, which can promote growth performance and health and are further used as antibiotic alternatives, is an important driver towards a sustainable diet for the livestock industry. To date, a precise protocol for the isolation and cultivation of chicken immune cells for the assessment of immunomodulatory functions of feed additives has not been reported. Therefore, the aim of this study was to establish a protocol for an *in vitro* cell culture system with avian PBMCs to assess the immunomodulatory effects of feed additives.

Methods: The optimal cell culture conditions of chicken PBMCs were established by testing different anticoagulants for blood sampling (200 µl 0.5 M EDTA (Roth), commercial heparin-, EDTA-, and citrate-coated tubes (Greiner Bio-One)), different PBMC isolation methods (slow-speed centrifugation-ficoll (Sigma-Aldrich), dextran (Roth)-ficoll (2)), as well as different sera as cell culture supplementation (porcine, fetal calf (FCS), and chicken serum). Furthermore, the effect of the supplementation of additional L-glutamine (Gibco) on the cell viability was tested. Moreover, antibody sets for immunophenotyping using flow cytometry for the differentiation of immune cell types were established. For validation of a response capacity towards immune stimuli, an addition of 10 µg/ml concanavalin A (conA) (Vector Labs) was used as a positive control. The cells were cultured in RPMI 1640 Medium (Gibco) with L-glutamine, sodium bicarbonate, and phenol red at 41 °C and 5 % CO2. Statistical analysis was performed based on a Student's t-test.

Results: The citrate tubes revealed the highest live cell count compared to EDTA ($p < 0.05$) and heparin ($p < 0.01$) tubes. Furthermore, the relative cell count of lymphocytes sampled in citrate tubes was higher compared to 200 µl 0.5 M EDTA ($p < 0.05$). The relative cell count of leukocytes was highest when blood was sampled in heparin compared to citrate ($p < 0.01$) and EDTA ($p < 0.01$) tubes. The relative thrombocyte count was highest in citrate tubes compared to heparin ($p < 0.01$) and 200 µl 0.5 M EDTA ($p < 0.1$) tubes. However, as citrate revealed the highest live cell count and is further often used for immunological studies, we used citrate tubes as the anticoagulant. The dextran-ficoll isolation decreased the relative thrombocyte count significantly ($p < 0.01$). For PBMC culture, the addition of chicken serum revealed the highest relative leukocyte count compared to the cultivation with FCS ($p < 0.05$) and the lowest relative thrombocyte count compared to FCS ($p < 0.05$) after 1 day of cultivation. The cell viability did not differ between the chicken serum and FCS. The supplementation of additional L-glutamine to the cells cultured in RPMI 1640 with chicken serum did not change the cell viability. ConA treatment revealed an activation of CD4 T-helper cells ($p < 0.05$) and CD8 cytotoxic T-cells ($p < 0.1$) and can serve as a positive control in the cell culture system.

Conclusion: After extensive optimization steps,an *in vitro* model with chicken PBMCs is established that can be used to investigate the effects of immunomodulatory functions of feed additives.

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87.

Corticosterone, free and bound cortisol, and metabolic adaptations during the early inflammatory response to an intramammary lipopolysaccharide challenge in dairy cows

Corticosteron, freies und gebundenes Cortisol, und Stoffwechselanpassungen während der frühen Immunreaktion auf eine Lipopolysaccharid-Challenge bei Milchkühen

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Glucocorticoids are important regulators of metabolic and inflammatory stress. The majority of glucocorticoids are either specifically or non-specifically bound to plasma proteins, i.e. corticosteroid-binding-globulin [CBG] and serum albumin, respectively. Only a small remaining fraction of free cortisol (FC) is considered to be biologically active after binding to intracellular receptors. Hence not only total cortisol (TC), but also FC concentrations increase during inflammatory stages. Concomitantly, an activated immune system requires a coordinated supply with nutrients that is mediated by insulin and glucagon. The objective of the present study was to investigate the contribution of corticosterone, total and free cortisol to the inflammatory response to an intramammary LPS challenge in dairy cows. Furthermore, we evaluated relationships of glucocorticoid changes with concomitant alterations of metabolic factors and their key regulatory hormones insulin and glucagon. **Methods:** Blood samples of 10 multiparous Holstein dairy cows (parity: 3.0 ± 1.0, previous lactation yield: 7,601 ± 938 kg, mean ± SD) on the day of an intramammary LPS challenge (26.8 ± 3.4 d in milk; 50 µg LPS from E. coli serotype O26:B6) were taken every 30 min up to 5 h after the LPS instillation, and rectal temperature and heart rate were measured. Total cortisol (TC), insulin and glucagon concentrations in plasma were measured with RIA. The proportion of free cortisol (FC) was measured at 0, 3.5, and 5 h relative to the LPS administration by ultrafiltration. The intra- and inter-assay CV for TC and FC determination were < 3.2% and < 6.5%, respectively. Corticosterone was measured by a commercially available EIA. Concentrations of plasma metabolites (glucose, non-esterified fatty acids [NEFA], and β-hydroxybutyrate [BHB]), and serum albumin were measured using commercially available kits. Statistical analysis was performed using mixed models with time as fixed effect and cow as repeated subject to evaluate differences within the investigated parameters between selected time points (0, 3.5, and 5 h relative to the intramammary LPS administration). In addition, for the intervals between 0 and 3.5 h (and 3.5 to 5 h relative to LPS administration, resp.). Pearson correlation coefficients between concentration changes of either TC, FC or corticosterone and the respective changes of other glucocorticoids, vital signs, plasma metabolites, serum albumin, insulin, and glucagon were calculated. Significant effects were considered at $P<0.05$. **Results:** Rectal temperature increased up to 41.6 ± 0.1 °C at 5 h after the LPS application. Concentrations of TC and corticosterone increased until 3.5 h, and the proportion of FC relative to TC more than doubled until 3.5 h after LPS administration. Serum albumin concentration was reduced at 5 h compared to initial values, whereas concentrations of insulin, glucagon, and glucose were increased after 5 h compared to 0 h. Concentrations of NEFA and BHB did not change during the experiment. As expected, particularly TC, FC, and corticosterone were highly correlated among each other, whereas significant correlations with metabolic and endocrine factors were less frequent. Between 3.5 and 5 h post challenge, alterations in TC concentrations were positively associated with rectal temperature by trend ($P=0.09$), and a greater increase of plasma corticosterone concentration during this interval was positively correlated with a greater increase in plasma glucose concentration ($P<0.05$), whereas FC was associated with plasma glucose concentration only by trend ($P=0.07$). A further trend towards a significant negative correlation was observed among corticosterone and heart rate between 0 and 3.5 h after the LPS administration ($P=0.08$). Changes in glucocorticoid concentrations were positively correlated with concomitant alterations of insulin and glucagon. **Conclusion:** The stimulation of the immune system by the intramammary LPS administration is accompanied by distinct metabolic and endocrine changes. Corticosterone and TC concentrations react similarly in response to the LPS challenge and earlier compared to metabolic adaptations. The increased need of active cortisol is covered by both increased secretion and a higher percentage of FC.

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88.

Estimation of energy digestibility from organic matter digestibility in cattle and sheep

Schätzung der Verdaulichkeit der Energie aus der Verdaulichkeit der Organischen Masse bei Rindern und Schafen

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Determination of energy digestibility (ED) of feedstuffs requires digestibility trials and subsequent calorimetry of feed and faeces. However, results of digestibility trials often do not include the information on ED. Furthermore, *in vitro*-measurements determine directly or indirectly organic matter (OM) digestibility (OMD). Therefore, the close relationship between OMD and ED might be used to enable the calculation of ED from OMD. The objective of the present study was to precisely assess the relationship between OMD and ED determined in metabolism experiments with cattle and sheep.

Methods: Two datasets were collated from results of metabolism experiments with cattle (1, 2, 3) and sheep (3) performed at the former Oskar Kellner-Institute, Rostock, Germany. Experimental diets displayed considerable variability in both diet components and chemical composition. Feeding level (FL) was 1.1 with a standard deviation (SD) of 0.20 for cattle and 0.9 (SD=0.13) for sheep, with FL=1 defined at 50 g dry matter (DM) intake per kg0.75 (metabolic body size). For both cattle and sheep, diets containing <100 or >200 g/kg DM of crude protein (CP) were excluded, as were diets containing >70 g/DM of ether extract. Taking these boundaries into account, 102 and 63 diets fed to cattle and sheep, respectively, were used. Mean OMD was 74.0% (SD=5.64%; range 56.6-83.9%) and 73.7% (SD=6.34%; range 58.3-84.9%) for cattle and sheep, respectively. Mean ED was 70.8% (SD=5.69%, range 53.0-81.5%) and 70.5% (SD=6.50%; range 54.6-82.1%) for cattle and sheep, respectively. Descriptive statistics and linear regression analysis were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) using procedures PROC MEANS and PROC REG, respectively. Fit statistics were given as coefficient of determination (R^2) and root mean squared error (RMSE).

Results: Regressions of ED on OMD were (i) ED = −3.63 + 1.01 OMD (R^2=0.99; RMSE=0.52) for cattle and (ii) ED = −4.66 + 1.02 OMD (R^2=0.99; RMSE=0.69) for sheep, with ED and OMD given as %. In both equations, slopes were not significantly different from 1. Consistently, linear regression showed no effect of OMD on differences (percentage points) between OMD and ED (OMD−ED) for both cattle and sheep. Therefore, a simple estimation of ED using mean OMD−ED (3.26, SD=0.516 for cattle; 3.19, SD=0.696 for sheep) could be considered. However, linear regression analysis revealed a decrease of OMD−ED with increasing dietary CP concentration (g/kg OM): (iii) OMD−ED = 5.04 − 0.0113 CP (R^2=0.31; RMSE=0.432) for cattle and (iv) OMD−ED = 5.45 − 0.0133 CP (R^2=0.26; RMSE=0.605) for sheep.

Conclusion: The observed effect of CP concentration on OMD−ED may be due to a higher caloric value of CP associated with higher digestibility of CP compared to carbohydrates. However, this effect may be negligible in standardised feed evaluation as the differences between SD of OMD−ED (the equivalent of RMSE when OMD−ED is fixed at its respective mean value) and RMSE of equations (iii) and (iv) were <0.1. Considering boundary CP concentrations of 100 and 200 g/kg DM for a given diet with OMD of 75.0% and 80 g ash /kg DM, equation (iii) would result in estimated ED of 71.2 and 72.4%, respectively, whereas assumption of 3.2 as constant OMD−ED would result in estimated ED of 71.8% regardless of CP concentration. The difference between ED estimated using equation (iii) or constant OMD−ED would be reduced at less extreme CP concentrations. Hence, for the use in standardised feed evaluation, that is at a FL of about 1, assuming a mean difference of 3.2 percentage points may be sufficient to estimate ED from OMD values for cattle and sheep. This conclusion is supported by the fact that OMD−ED was not affected by OMD. Although diets were not congruent, very similar results for cattle and sheep indicate that the use of OMD from sheep digestibility trials to estimate ED in cattle feed is valid.

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89.

Does the increase of C12:0 in hair during early lactation show a lower energy deficit?

Zeigt die Erhöhung des C12:0 Gehalts im Haar während der frühen Laktation ein geringeres Energiedefizit an?

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Previously, we have shown that primiparous cows with a short interval from calving to conception and a high milk protein yield (1) as well as cows with a high energy utilisation in lactation weeks 1 to 6 (2) had higher contents of lauric acid (C12:0) in hair in lactation week 8. The aim of this study was to test the association between the difference of C12:0 contents in hair between lactation week 4 and 8 and the energy balance during lactation weeks 4 to 6.
Methods: For the study, 27 and 34 Simmental and 34 German Holstein cows from three experimental farms were used. All farms were equipped with feeding systems to measure individual daily feed intake. The lactation number varied from 2 to 9. All cows were fed two levels of energy concentration of roughage (6.1 and 6.5 MJ NEL/kg DM) and two levels of amount of concentrates (150 and 250 g/kg ECM). Due to the low number of animals per feeding group, feeding group was not considered for further analysis. Hair samples were taken from each cow in week 4 and 8 of lactation. Hair lipids were extracted from 200 mg cleaned and mill-ground hair with a fatty extraction kit (3). After methylation of fatty acids, the fatty acid methyl esters were analysed using gas chromatography. Cows were classified according to their difference in C12:0 contents from week 4 to 8 in two groups: (A) increase (difference > 0) or (B) decrease of C12:0 (difference < 0) content. The energy balance (MJ ME/d) was defined as the difference between energy intake and energy requirements for maintenance and milk production. Since the fatty acids in hair of an animal reflect the metabolism 2 to 3 weeks before, we calculated the energy balance for the period from the first shaving in week 4 to week 6. To evaluate the influence of increased and decreased C12:0 content in hair on the energy balance, an analysis of variances was performed for each farm including the C12:0 group, lactation week (4, 5, 6) and their interaction as fixed effects into the model. Lactation week was used as repeated measurement statement.
Results: The average differences between the C12:0 contents in week 4 and 8 in the increasing and decreasing groups were 0.8 ±0.6% (n=14) and -0.4±0.4% (n=13) in farm 1 (SIM), 0.7 ±0.6% (n=23) and -0.7±0.7% (n=11) in farm 2 (SIM), and 0.5 ±0.4% (n=18) and -0.4±0.4% (n=16) in farm 3 (HOL), respectively. The average daily energy balance (MJ ME) during the lactation weeks 4 to 6 was negative for all groups. But in all three farms, the cows increasing their C12:0 content in hair during early lactation had a better energy balance during this period, which means the energy deficit was lower. The energy balance was 28 MJ ME/d ($P < 0.05$) and 17 MJ ME/d ($P = 0.05$) higher in the C12:0 increasing compared to the decreasing group in the SIM farms 1 and 2, respectively, and 9 MJ ME/d ($P > 0.05$) higher in the HOL farm.
Conclusion: The results provide further evidence that the amount of the de novo synthesised fatty acid C12:0 in the hair of lactating cows is linked to the energy availability of a cow. An increase of the C12:0 content in the hair of cows during lactation weeks 4 to 8 is associated with a better energy balance in lactation weeks 4 to 6. This leads to a lower energy deficit in the early lactation.
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90.

Effects of 3-Nitrooxypropanol and varying concentrate feed proportions in the ration on rumen volatile fatty acids and hepatic function parameters in peripartal dairy cows

Einfluss von 3-Nitrooxypropanol und variierendem Kraftfutteranteil in der Ration auf flüchtige Fettsäuren im Pansen sowie Parameter der Leberfunktionalität bei peripartalen Milchkühen

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The climate-relevant ruminal methanogenesis causes a loss of gross energy intake but is a major H_2 sink. 3-Nitrooxypropanol (3-NOP) inhibits the last step of CH_4 formation (1). Transitional cows are challenged with energy demands for milk energy excretion exceeding energy intake which may cause exaggerated mobilization of triglycerides from adipose tissues resulting in liver lesions. We hypothesized that higher concentrate feed proportion (CFP) and 3-NOP induce a shift to alternative H_2 removing fermentation pathways in favor of glucogenic VFA in a synergistic manner. This could reduce the peripartal energy deficit and, therefore, a metabolic hepatic overload and consecutive hepatocellular lesions. **Methods:** From d 28 antepartum (a.p.) until d 120 postpartum (p.p.), 55 pluriparous German Holstein cows were grouped in a 2x2 factorial design by low (CL) or high (CH) CFP tested without supplements (CONCL, CONCH) or combined with 3-NOP (NOPCL, 48.4 mg/kg DM; NOPCH, 51.2 mg/kg DM) in the ration. 3-NOP was supplied via concentrates from both the partial mixed ration (70% maize silage, 20% grass silage, 10% concentrates on DM basis) and additional concentrates provided by automatic feeders. Before calving, CL and CH groups received a CFP of 15% and 40%, respectively. From parturition until d 21 p.p., CFP of CH groups gradually increased from 30 to 55%. CFP of CL groups was maintained at 30% after parturition. On d 28, 14, 7 a.p. and d 7, 28, 49, 73, 98, 120 p.p., rumen fluid was collected by using an oro-ruminal probe and suction pump to analyze VFA. Serum samples were prepared from jugular vein blood for additional time points on d 3 a.p. and d 1, 3, 14, 21, 35 p.p. to determine liver enzyme activities and metabolite concentrations photometrically. Liver biopsy was conducted on d 28 a.p. and d 7, 28, 120 p.p. to determine total lipid content (TL) in liver tissue. Statistics were performed (PROC MIXED SAS v9.4) with 3-NOP, CFP, time relative to parturition (TIME), and their interactions as fixed effects, cow as random effect and sampling day as a repeated measure. **Results:** Effects of the fixed factors on milk and CH_4 production, depot fat mobilization and ketone bodies were reported in (2,3). 3-NOP and high CFP decreased molar proportion of acetate in rumen fluid (3-NOP×TIME; $p<0.01$; CFP×TIME; $p<0.01$) but increased that of propionate, butyrate, valerate (3-NOP; $p<0.01$; CFP×TIME; $p<0.01$) and iso-valerate (3-NOP×TIME; $p=0.007$). Iso-butyrate was unaffected by 3-NOP but decreased in CH groups. Rumen total VFA and TL in liver tissue were only affected by TIME. 3-NOP and high CFP significantly decreased alanine transaminase activity, whereby that of aspartate transaminase decreased in CL and 3-NOP groups being most apparent from d 7 until d 35 p.p. (3-NOP; $p=0.018$; CFP×TIME; $p=0.039$). 3-NOP temporarily reduced albumin and bilirubin concentration (3-NOP×TIME; $p<0.05$), whereby the latter also decreased in CH groups (CFP×TIME; $p=0.045$). Glutamate dehydrogenase and γ-glutamyl transferase activity remained unaffected by 3-NOP but increased in CH and decreased in CL groups from d 49 p.p. to d 120 p.p. (CFP×TIME; $p<0.05$). **Conclusions:** We confirmed our hypothesis that high CFP and 3-NOP in the diet increased glucogenic VFA and partially reduced liver lesion indicating enzyme activities in blood. Synergistic effects of CFP and 3-NOP on measured parameters were not consistently observed.

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91.

Effect of Acacia mearnsii supplementation on ruminal fermentation characteristics and methane production of dairy cows fed diets differing in silage type

Einfluss der Zufütterung von Acacia mearnsii zu Rationen auf Basis unterschiedlicher Silagen auf die Pansenfermentation und die Methanproduktion von Milchkühen

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Feeding forage-based diets to ruminants permits to valorize lands unsuitable for intensive cultivation, converting low nutritional value feed into food with high nutritional value. Nevertheless feeding high proportions of forage increases ruminal methane (CH_4) emissions due to the high content of fibre in the cell walls of the forages. Dietary supplementation of a tannin-rich extract from Acacia mearnsii bark can reduce CH_4 production (1). This study investigates the efficiency of A. mearnsii to mitigate ruminal CH_4 emissions in three diets differing in silage type and crude protein (CP) content.

Methods: In a 3 × 6 incomplete Latin Square design, 30 Holstein cows (milk yield: 33.8 ± 7.6 kg/d; 63 ± 23 d in milk) were blocked by milk yield and assigned randomly to six treatments. Each experimental period lasted 28-d including 15-d adaptation and 13-d data collection period. Cows received one of six total mixed rations at ad libitum access that contained (on dry matter (DM) basis) 79% silage and 21% concentrate. The silages tested were either ryegrass-based (R, 13% CP), red clover-based (C, 20% CP) or tanniferous sainfoin-based (S, 17% CP). In addition, diets were supplemented at 2.1% either with an extract rich in tannins prepared from the bark of A. mearnsii (Baeck, Norderstedt, Germany) or with straw meal. Intake of dry matter (DMI) and fiber (NDFI) and milk yield were recorded daily, and milk composition once a week. The CH_4 production of each cow was determined using the GreenFeed© system (C-Lock Inc., Rapid City, SD, USA). Ruminal fluid was collected twice per collection period using a stomach tube and was analyzed for the standard parameters. Data were analyzed across the 13-d collection period using linear mixed models with type of silage, tannin supplementation and their interaction as fixed factors.

Results: Tannin supplementation caused a decrease in DMI (19.8 kg/d vs 20.4 kg/d) and NDFI ($P < 0.001$) but this effect was not consistent among the different silage types (tannin × silage interaction, $P < 0.01$). Cows fed C had the highest ($P < 0.01$) DMI and NDFI, followed by cows fed S and R for DMI and R and S for NDFI. Tannin-supplemented cows produced less ($P<0.01$) milk (26.2 vs 27.5 kg/d) and energy corrected milk (ECM; 26.6 vs 28.3 kg/d) than straw-supplemented cows. Daily production of milk and ECM was highest ($P < 0.05$) with C (29.4 kg/d milk; 29.8 kg/d ECM), followed by S (26.5 kg/d milk; 26.5 kg/d ECM) and R (24.8 kg/d milk; 25.9 kg/d ECM). Milk urea was lower ($P < 0.01$) in tannin-supplemented (142 mg/kg) than straw-supplemented cows (170 mg/kg). Cows fed C (259 mg/kg) had the highest ($P < 0.001$) milk urea content followed by cows fed S (135 mg/kg) and R (74 mg/kg). The same effect of silage type ($P < 0.001$) was observed for ruminal ammonia concentration. Tannin supplementation caused an increase ($P < 0.001$) in the proportion of ruminal propionate and butyrate at the expense of acetate. Cows fed C and S had a higher ($P < 0.001$) proportion of acetate and a lower ($P < 0.001$) proportion of propionate, and butyrate compared to cows fed R. Daily CH_4 production and production related to DMI were lower ($P < 0.001$) in the tannin-supplemented cows (359 g/d; 18.4 g/kg) compared to straw-supplemented cows (398 g/d; 19.8 g/kg). Cows fed S (364 g) and R (372 g) produced less ($P < 0.05$) CH_4 per day compared to cows fed C (400 g) whereas, related to DMI S (18.1 g/kg) and C (18.4 g/kg) cows produced less ($P < 0.05$) CH_4than R cows (20.7 g/kg). No effects ($P > 0.05$) of tannin supplementation and silage type were observed on CH_4 production related to NDFI and ECM (tannin-supplemented: 13.7 g/kg; straw-supplemented: 14.7 g/kg; R: 14.5 g/kg; C:13.6 g/kg; S: 13.9 g/kg).

Conclusion: Independent of silage type, supplementation of Acacia mearnsii was efficient in reducing CH_4 production per day and related to DMI but not related to ECM and NDFI. While the CH_4 reducing effect of ryegrass and red clover seemed to be influenced by DMI, feeding of sainfoin silage caused a decrease in methane production both per day and related to DMI.

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92.

Comparison of tanniferous wood extracts in their effect on methane production of dairy cows

Vergleich von tanninhaltigen Holzextrakten hinsichtlich ihrer Wirkung auf die Methanproduktion von Milchkühen

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Among the various greenhouse gases produced by livestock husbandry, enteric methane (CH_4) from ruminants is the most significant contributor. For long, research on CH_4 mitigation has been focused on nutritional strategies, of which the feeding of tannins is a promising one. Tannins are a heterogeneous group of phenolic compounds. Due to their chemical properties they can be divided into hydrolysable and condensed tannins that differ regarding the magnitude of CH_4 decrease they produced *in vitro* (1). The aim of the present study was to compare the effect of bioactive plant extracts differing in the level of hydrolysable and condensed tannins on ruminal methane production of dairy cows.
Methods: A feeding experiment split into a three week pre-treatment period, and a five week experimental period (EP) was conducted with 30 Holstein cows (number of lactation: 2.2 ± 1.2; days in milk: 77 ± 28 d; milk yield: 35.1 ± 5.6 kg/d). Cows were paired by number and stage of lactation and milk yield and assigned randomly to two treatments. Cows were fed a total mixed ration (TMR) containing hay, corn silage, grass silage and concentrate (33:28:26:13%). In the experimental period, the ration was supplemented at 1.2% of the total diet either with a chestnut extract (Silvafeed Nutri P, Ledoga s.r.l., San Michele Mondovi, Italy) mainly containing hydrolysable tannins (CE), or with a quebracho extract (Silvafeed Bypro Q, Ledoga s.r.l) mainly containing condensed tannins (QE). Feed intake and milk yield were recorded daily and milk components were analysed weekly. The CH_4 production was measured during visits to a GreenFeed system (C-Lock Inc., Rapid City, SD, USA). Visits were encouraged by offering small portions (33 g) of bait feed (pelleted dried whole maize plant). Daily CH_4 production, milk yield and feed intake data were averaged per cow per experimental week. Data were analysed with the package lme4 of R software using linear mixed models with treatment, experimental week and their interaction as fixed effects, cow as random effect and averaged data of the pre-treatment period as covariate.
Results: Daily intake of dry matter (DM; 21.7 kg) and neutral detergent fibre (NDF; 8.20 kg) did not differ between treatment groups ($P > 0.05$) but varied differently for treatments across experimental weeks (interaction effect $P < 0.01$). Cows fed QE (30.6 kg/d) produced less milk ($P < 0.05$) compared to cows fed CE (32.2 kg/d). Milk yield decreased for both treatments during the EP ($P < 0.001$). Neither milk fat (4.33%) and protein (3.42%) nor lactose (4.84%) were affected ($P>0.05$) by treatment. Milk protein increased ($P < 0.001$) for both treatments during the EP. Although there was an effect of experimental week ($P < 0.05$) on milk fat and lactose, no clear trend across the experimental weeks was observed. Daily CH_4 production was lower ($P<0.05$) for cows fed QE (441 g) compared to cows fed CE (451 g). Treatment differences varied across experimental weeks (interaction effect $P <0 .001$) in a way that CH_4 production in the QE group was stable in week one to three and decreased in week four and five whereas in the CE group CH_4 production was low in the first two weeks and increased in week three, four and five. Neither CH_4 production per kg DM intake nor per kg NDF intake was affected by treatment or experimental week ($P > 0.05$). Methane intensity (production per kg ECM) was higher ($P < 0.01$) for cows fed QE (14.0 g) compared to cows fed CE (13.5 g). For the QE group, CH_4 intensity was similar across experimental weeks whereas for the CE group the CH_4 intensity was lower in week one and two and highest in week five (interaction effect $P < 0.001$).
Conclusions: The supplementation of quebracho extract to a TMR had a small but significant decreasing effect on daily CH_4 production of dairy cows compared to the supplementation of chestnut extract. However, the effect was accompanied with a decrease in milk production that resulted in a higher CH_4 intensity for cows fed quebracho extract.

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93.

Intestinal integrity characteristics of growing pigs in response to oregano essential oil supplementation

Darmintegritätsmerkmale von Mastschweinen in Reaktion auf die Supplementierung von Oreganoöl

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Pig production depends on a health and performance balance. An approach to improve intestinal health is the oregano essential oil (OEO) supplementation within a conventional diet. Intestinal integrity regulating effects, e.g. gene expression, of some feed ingredients are important key factors for that balance. We hypothesized that OEO from Origanum Vulgare subsp. Hirtum affects the expression of genes associated with pigs´ immune system and regulates the cell activity in the small intestine.

Methods: To evaluate the influence of OEO on pigs´ genetics, carcass quality, and blood profile, we used pigs with low genetic variability. For this, nine gilts (German Landrace, siblings) have been paired with one Pietrain boar. In four similar trials, a total of 86 pigs were used. In each trial, pigs´ have been equally divided into two groups. Groups were kept under identical conditions with maximal four pigs per pen and one exception. The ´treated´ group was additionally fed an oregano flavour additive (DOSTO® powder) within the basal diet. The concentration of the flavour additive within the diet was 1500 mg/kg (7.5% pure OEO). During fattening, blood samples have been taken at four different time points. Complete blood counts were generated by flow cytometry technique. At age of 6 months, pigs were slaughtered with an average weight of 111.1±10.9 kg. In addition to automatically generated Auto-FOM data, carcass quality factors, e.g. pH, conductivity, drip loss, meat composition, etc., have been measured manually. For transcriptome analysis 12 homogenous pigs (104.3±2 kg, one sow, evenly distributed sexes and groups) have been selected from one trial to eliminate seasonal effects. Converted RNA probes were injected into the GeneChip Porcine Gene 1.0 ST array and scanned by GeneChip scanner 3000 7G. Probe to gene transcript annotation was performed with a common Affymetrix annotation file (PorGene-1_0-st-v1)1. Transcriptional modifications investigation in response to feeding regime and the differential gene expression analysis was performed by using the ´Linear Models for Microarray Data (limma)´ technique (v. 3.44.3)2 with empirical Bayes adjustment to the variance, followed by Benjamini and Hochberg correction for multiple testing. Pairwise contrast for treated vs. control groups were considered for differential gene expression. For topTable generation, the adjusted p-value has been set to <0.3. Microarray expression results were technically validated by qPCR within a higher number of animals (n=36). Relative expression was calculated with 2-ΔΔCT method for qPCR.

Results: Performance data can be used to describe nutritional effects on animal health. As a result of this investigation, few significant differences according to animal development and meat quality have been found as compared to the OEO supplementation. Neck fat tended to be thicker in control animals ($p<0.08$) and weight of ham and loin cuts was significant lower ($p<0.05$) in the treated group. We found no significant differences in meat quality characteristics, e.g. percentage of collagen, fat, protein and water, between the groups. In general, we found very limited effects on pigs´ haematology. At the beginning of the feeding trial (weaning, age 28 days) we found significant reduced ($p<0.05$) haemoglobin, haematocrit, MCV, and MCH values. Whereas, band value was significant increased ($p<0.05$) in the treated group at this time point. Depending on OEO supplementation, we found 93 differently regulated genes in the jejunal tissue (70 up, 23 down) and 60 in the ileal tissue (48 up, 12 down). The gene expression between both investigated small intestinal tissues was significant ($p<0.001$) different. Just three genes (GRIN3B, ZO-1, and one uncharacterized gene) were affected by OEO in the jejunum, as well as in the ileum. qPCR validation revealed AKT3, Interferon-ε, -ω, ZO-1 to be up-regulated in the jejunum and CCL21 was up-regulated in the ileum

Conclusion: OEO supplementation had limited effects on pigs´ performance traits and haematology. However, OEO supplementation has several effects on the expression of genes associated with intestinal integrity in the pigs´ small intestine. These findings help to understand the nutritional impact on intestinal health.

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94.

Comparison of three indigestible markers (acid-insoluble ash, Cr_2O_3, TiO_2) used in digestibility studies in pigs – a technical note

Methodische Untersuchung dreier Marker (HCl-unlösliche Asche, Cr_2O_3, TiO_2) für Studien zur Nährstoffverdaulichkeit beim Schwein

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Indigestible markers are tools for digestibility studies, in particular when a total collection (TC) of chyme or faeces is not feasible. This problem particularly occurs when determining apparent ileal digestibility (AID) by using the ethically approved simple T-cannulation. In previous studies with T-cannulated growing pigs, acid-insoluble ash (AIA) was used as marker, but with rather variable results at ileal, but not at faecal level. As the aim was to determine AID and apparent total tract digestibility (ATTD) in the same animal, reliable marker are required. Thus, the objective of this study was to evaluate the suitability of three commonly used markers in determining AID and ATTD in pigs.
Methods: Three barrows (118.9±4.4 kg LW), each fitted with a T-cannula at the terminal ileum and adapted to individual housing in flatdeck-units, were used. During a 7d adaptation period pigs were housed in floor pens and thereafter in flatdeck-units with slatted floors for a 7d-collection. Pigs received a barley-based diet (2 kg/pig*d), consisting of 90% barley, 5% high-protein soy bean meal, 2% soya oil, 5.29% premix and supplemented with three markers: 1% SiO_2 (AIA), 0.5% titanium dioxide TiO_2 (TiO) and 0.1% chromium oxide Cr_2O_3 (Cr). During the 7d-collection period, urine and faeces were collected quantitatively for each pig. On day 8 and 9, ileal digesta was collected via the T-cannula and pooled for 24h. Formic acid (2.5M) was added to each matrix during collection preventing microbial nitrogen conversion. Nutrient and AIA concentrations were analysed following standard VDLUFA procedures. The concentrations of Cr and TiO were determined by ICP-OES. Faecal recovery of indigestible markers was calculated based on TC. ATTD of organic matter (OM), protein, fat, N-free extracts (NfE) as well as metabolisable energy (ME, MJ/kg) were determined based on TC and marker methods. AID of nutrients were calculated based on the three markers only. Statistical evaluation with "marker" as fixed factor was carried out using proc mixed and an adjusted Tukey-test as post-hoc procedure (SAS 9.4). Data are presented as means±SD.
Results: The TC method was utilised as a benchmark method, comparing nutrient digestibility to those derived from indigestible markers. In general, ATTD calculated from TC method was significantly higher than those derived from indigestible markers ($p<0.05$). ATTD based on AIA was significantly higher for all analysed nutrients than those derived from TiO ($p<0.05$), whereas Cr-based data did not differ significantly from the other two markers. In example: ATTD of OM was calculated at 88.2±1.1% for TC, 85.7±0.9% for AIA, 84.3±2.0% for Cr and 83.5±1.2% for TiO and thus represented 97%, 96% and 95% of the TC-obtained data for AIA, Cr and TiO, respectively. The total faecal recovery (% of intake) of each indigestible marker was calculated and appeared highest for AIA (82±3%), followed by Cr (75±4%) and TiO (72±4%). AID was determined based on indigestible markers as a TC method is not feasible with T-cannulated pigs. Here, contrasting results to ATTD were observed with respect to the employed marker: AID of OM was calculated at 62.4±3.7% for AIA, 74.4±2.5% for Cr and 75.8±2.7% for TiO. Thus, AID derived from AIA were significantly lower by ~20% as those calculated from Cr and TiO ($p<0.05$), whereby the latter two did not differ from each other.
Conclusions: In the present study, AIA showed superior faecal recovery as compared to Cr and TiO and thus yielded only slightly lower ATTD digestibility data compared to the benchmark method of TC. In contrast to this, ileal digestibility was persistently lower when employing AIA rather than Cr or TiO. This raises the question whether ileal digestibility based on AIA is underrated or those based on Cr and TiO are overrated? Because we are currently lacking an ethically approved benchmark method for AID enabling total digesta collection, we cannot answer this issue satisfactorily.

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95.

Retention and precaecal digestibility of guanidinoacetic acid and creatine in minipigs

Verwertung und präzäkale Verdaulichkeit von Guanidinoessigsäure und Kreatin bei Minipigs

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Creatine (Cr) is an essential biomolecule for cellular energy homeostasis. It is endogenously produced in all vertebrates from arginine and glycine via guanidinoacetic acid (GAA) as intermediate step. Cr is stored in high amounts in skeletal muscle. Hence, Cr is naturally supplied through meat and meat products. As omnivorous species, pigs' original diet consists of roots, grains, insects, worms and small mammals. Thus, they are evolutionary designed to obtain Cr from their diet. As modern swine feeding programs are primarily based on plants and contain only little animal derived proteins, pigs rely to a high extent on endogenous Cr synthesis. Supplementation of Cr to humans or GAA as a source of Cr to animals increases the muscle Cr contents resulting in more lean body mass and supporting muscle performance (1). In humans, per day approximately 1.7% of the total Cr pool are lost via formation of the degradation product creatinine (Crn) (2). GAA is in use as a source of creatine for pigs and poultry. In broilers, GAA showed a true faecal digestibility of close to 100% in a dose range of 0.06%-0.60% (3). Therefore, its digestibility and retention was determined in miniature pigs as well.

Methods: Four individually housed adult ileo-rectal anastomised male-castrated minipigs (Minilewe) (initial bodyweight 71.8 ± 6.4 kg, final bodyweight 74.6 ± 5.2 kg) received five test diets in a latin-square-like trial design. The test diets were CON (wheat (27%), maize (24%), soybean meal (23%), and barley (20%)), CON + either 0.06, 0.12, or 0.60% GAA, and CON + 0.15% Cr. All animals received the CON diet during the 10 days initial period. Afterwards, the four test diets were fed for 5 days each separated by a 5 days adaption period. During the test periods, urine and chyme samples were collected daily, weighed and freeze-dried for further analyses. All feeds were produced and sampled for nutrient analyses on the same day. Individual daily feed intake was recorded. Cr, Crn, GAA and amino acids in feed, urine and chyme were measured for calculation of apparent and true digestibility and retention of GAA, Cr, and amino acids. True digestibility and retention were obtained via correction by endogenous losses during the initial period. All three Cr-metabolites (GAA, Cr and Crn) were summed for retention calculation. Statistical analyses comprised a one-factorial ANOVA (Cr-source as independent variate). Significant differences were identified using the Student-Newman-Keuls test.

Results: Numerically, the true digestibility of GAA decreased with increasing GAA dose from 96.6% (0.06% GAA) over 94.4% (0.12%) to 90.4% (0.60%). The true digestibility of Cr was 96.3%. In unsupplemented condition excretion of GAA, Cr and CRN was higher than intake of the respective nutrients, thereby resulting in a still negative apparent retention in the low GAA and Cr diets. The addition of Cr sources generally increased the apparent retention and became positive with the supplementation of 0.60% GAA indicating a reduced need for endogenous synthesis. The true retention was on similar levels of ~1300 mg/pig and day at 0.06% and 0.12% of GAA and 0.15% Cr, and was significantly higher (3657 mg/pig and day) when supplemented with 0.60% GAA. Up to 0.12% GAA and 0.15% Cr all products of Cr-metabolism were primarily excreted via urine. Only the 0.60% dose led to increased excretion via chyme. The addition of any source of Cr had no significant effect on protein/amino acid digestibility.

Conclusions: Generally, GAA and Cr are highly digestible (>90%) in miniature pigs. The supplementation of 0.06% and 0.12% GAA supported the endogenous Cr synthesis. The 0.60% dose induced an oversupply with increased excretion of GAA, Cr and Crn in urine and chyme. This suggested saturation of all Cr metabolic pathways. This study indicates that the ideal dose of GAA in minipigs ranges between 0.06% and 0.12% which should further be elaborated in piglets, growing/finishing pigs, and/or sows.

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96.

Free faecal water: Faecal parameters determined in affected and healthy horses

Kotwasser: Kotparameter betroffener und unauffälliger Pferde

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Free faecal water is frequently reported in horses, especially in warmblood breeds. Although in the majority of cases not accompanied by severe health issues, this condition is generally regarded as a problem by the owners who often expend much effort and money on treatment, dietary supplements, etc. This study aimed to compare faecal parameters of affected and unaffected horses in three different riding stables.

Methods: In total, we sampled 31 horses from three different stables (A: 15; B: 10; C: 6) including 16 control animals (Con; A: 7; B: 6; C: 3) and 15 individuals presented with free faecal water (FFW; A: 8; B: 4; C: 3). In stable A, horses were kept on grass paddock 24/7, grass hay was fed ad libitum and concentrate was only supplied by the owners if necessary. In stable B, the animals were kept on grass paddock and received additional hay (3 kg) and concentrate once per day, whereas time spent outside was restricted to 7 hours and hay (5 kg) and concentrate were fed twice daily in stable C. Exact amounts and compositions of the concentrates differed between the individual animals. Faecal water content, pH, concentrations of short chain fatty acids (SCFA; acetate, propionate and butyrate) and lactate were determined by standard procedures or as described before (1,2). For a subpopulation of 8 horses from stable A, we additionally performed wet sieving to characterize the particle size distribution in faeces (> 8 mm, > 4 mm, > 2 mm, > 1 mm, > 0.5 mm, > 0.125 mm, > 0.063 mm; < 0.063 mm) as described by Hummel et al. (3). Data were analysed using a two-way ANOVA for the factors stable and FFW followed by Sidak´s multiple comparisons test and linear regression analysis (GraphPad Prism 8.4.3). Differences were considered significant at $P < 0.05$, results are presented as predicted means.

Results: Faecal water content amounted to 81.0 ± 1.45% on average and was affected neither by the stable nor by the occurrence of FFW. Faecal pH was influenced by the stable ($P < 0.001$; A: 6.82, B: 6.62, C: 6.28). In addition, FFW horses showed significantly greater pH values than Con animals ($P < 0.05$; FFW: 6.70, Con: 6.46) although lactate concentrations, for which we found a weak negative association with faecal pH ($P < 0.01$; r2: 0.27) were not altered. The proportion of acetate in relation to total SCFA differed among the three stables ($P < 0.05$; A: 78.4%, B: 76.9%, C: 75.3%). Interestingly, an interaction of stable and FFW was observed for the absolute concentration of acetate ($P < 0.05$) that was only higher in affected horses kept at stable A (FFW: 23.7 mmol/L, Con: 18.5 mmol/L). Particle size distribution did not seem to be associated with FFW.

Conclusions: The reason for the differences in faecal pH and the relative proportion of acetate between the three stables can be related to the management and the roughage intake. As neither lactate nor SCFA concentrations explain the higher pH observed with FFW, it can be speculated that secretion patterns, e. g. of buffers like phosphate or bicarbonate, are directly or indirectly altered in affected horses. Most important, the inconsistent results in respect to the absolute concentration of acetate underline the complexity of absorption and secretion mechanisms and their interaction with dietary factors that can complicate the interpretation of faecal parameters and the establishment of reference values.

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97.

Phytogenic feed additive modulates chewing behaviour, rumen fermentation and ruminal pH in dairy cows fed high grain diets

Ein phytogener Futterzusatzstoff beeinflusst das Kauverhalten, die Pansenfermentation und den Pansen-pH bei kraftfutterreich gefütterten Milchkühen

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Intensive dairy production systems feed large amounts of concentrates to support animal performance. A previous study by our research group has showed that a mixture of phytogenic (PHY) compounds increase daily mean reticular pH in dairy cows. Nevertheless, there is a lack of information regarding the effect of PHY compounds during an acidogenic diet challenge on chewing behavior and rumen fermentation parameters. Therefore, this experiment was conducted to evaluate the effects of a PHY compound on chewing activity, SCFA profile and pH in the rumen of non-lactating dairy cows. We hypothesized that PHY supplementation will improve chewing behaviour and modulate rumen fermentation.

Methods: Nine rumen cannulated non-lactating Holstein cows, in a cross-over design, were blocked by body weight (n = 5 and n = 4) and were supplemented with either a blend of phytogenic compounds that include menthol and thymol or a neutral control carrier in powder form, and first combined with the concentrate portion of the TMR. The experiment consisted in two experimental runs, in each run cows received a 100% forage diet (45% grass silage, 45% corn silage, 10% hay) for a week, then transitioned step-wise to 65% DM concentrate (high grain diet including 26.3% grass silage, 8.7% corn silage and 65% concentrate) over eight days. The high grain diet was fed for 4 weeks. Between the two experimental runs, there was a washout period of 3 weeks where cows grazed on pasture and received hay supplementation. Noseband sensor halters (RumiWatch System, ITIN +HOCH GmbH, Fütterungstechnik, Liestal, Switzerland) were used to monitor chewing activity for a minimum of 3 days each experimental week. Short chain fatty acid analysis was conducted on rumen fluid collected at 0 h (before feeding), 4, 8 and 12 hours (after feeding) using gas chromatography. Ruminal pH was continuously recorded at 15-minute intervals using indwelling pH measurement systems (Dascor Inc., Escondido, USA) placed in rumen ventral sac. Data were analysed with Proc Mixed from SAS with cow as random effect and week and treatment as fixed effect. Data collected on the first week (100% forage) was used as covariate for chewing behaviour.

Results: Eating time and ruminating time were not affected by the PHY feed additive. Nonetheless there was a week effect showing a reduction in eating time from week 1 (100% forage) to weeks 2-5 (high grain diets) ($P < 0.05$). Similarly, ruminating time was 397, 280, 290, 290, and 270 minutes per day on weeks 1 to 5, respectively ($P = 0.75$), but there was a reduction on ruminating time from the 100% forage to the high grain diet ($P < 0.05$). Total SCFA concentration did not show a treatment effect, but it increased from week 1 to week 5. Total SCFA concentrations were 89 and 115 mM for weeks 1 and 5, respectively. Acetate to propionate ratio increased with the PHY feed additive compared to control on week 3 with values of 2.5 vs 1.9 ($P < 0.05$). Additionally, there was an interaction effect between PHY and week for mean ruminal pH on weeks 4 and 5 with 6.03 vs. 6.15 ($P < 0.05$), and 5.99 vs. 6.12 ($P < 0.05$); for control and PHY feed additive, respectively.

Conclusion:. The phytogenic feed additive had a positive impact on rumen fermentation profile and may increase mean daily ruminal pH after 2 weeks of high grain feeding. Rumination and eating time were only affected by the diet composition.

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98.

Determination of ruminal protein degradation of concentrate feeds in situ and *in vitro*

Bestimmung des ruminalen Proteinabbaus aus Einzelfuttermitteln in situ und in vitro

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Estimation of ruminal protein degradation is an essential part of feed protein evaluation. Enzymatic *in vitro* methods such as the Streptomyces griseus protease test are potentially useful for routine analysis and do not rely on rumen-cannulated donor animals. The objective of this study was to assess the suitability of the S. griseus protease test for estimating effective degradation (ED) of protein from concentrate feeds compared with in situ degradation.

Methods: The following concentrate feeds, for which in situ results are available (1), were used: soybean meal (SBM), sunflower meal (SFM), rapeseed meal (RSM), over-toasted RSM, faba beans, maize, barley and dried distillers' grains with solubles (DDGS). In situ and *in vitro* investigations were performed on the same batches of evaluated feedstuffs. The S. griseus protease test was conducted according to Licitra et al. (2). In brief, duplicates of each 0.5 g of ground material were incubated for 1 h at 39 °C in 40 mL of borate-phosphate buffer (pH 6.7) under continuous shaking before the protease solution (0.58 U/mL) was added at a ratio of 24 U/g feed true protein (TP). TP was determined according to the CNCPS as crude protein (CP) minus non-protein nitrogen (fraction A). Soluble protein was determined as A plus TP soluble in borate-phosphate buffer (fraction B1). Incubation times were adapted to reflect the in situ trial and were: 0, 2, 4, 6, 8, 16 and 24 h. In addition, in situ measurements were obtained after 48 and 72 h of incubation. After incubation, the solutions were filtered and residues dried and analysed for Kjeldahl nitrogen. Protein degradation parameters were estimated following Ørskov and McDonald (3) and used to determine ED (%) for assumed ruminal passage rates of 0.05/h (ED5) and 0.08/h (ED8). The data was analysed by t-test at $P < 0.05$ significance level.

Results: Concentrations of CP (g/kg dry matter), TP (% of CP) and soluble protein (% of CP) were 479, 97 and 11 (SBM), 321, 92 and 36 (SFM), 358, 92 and 19 (RSM), 366, 92 and 15 (over-toasted RSM), 267, 87 and 55 (faba beans), 87, 87 and 18 (maize), 130, 88 and 34 (barley) and 317, 80 and 21 (DDGS). ED5 in situ vs. *in vitro* was 66 vs. 75 (SBM; $P < 0.001$), 79 vs. 77 (SFM), 69 vs. 60 (RSM; $P < 0.05$), 55 vs. 52 (over-toasted RSM; $P < 0.05$), 92 vs. 66 (faba beans; $P < 0.001$), 70 vs. 26 (maize; $P < 0.01$), 86 vs. 42 (barley; $P < 0.001$) and 82 vs. 50 % (DDGS; $P < 0.001$). ED8in situ vs. *in vitro* was 55 vs. 71 (SBM; $P < 0.001$), 72 vs. 74 (SFM), 61 vs. 55 (RSM; $P < 0.01$), 46 vs. 47 (over-toasted RSM), 89 vs. 65 (faba beans; $P < 0.001$), 61 vs. 25 (maize; $P < 0.01$), 82 vs. 41 (barley; $P < 0.001$) and 78 vs. 46 % (DDGS; $P < 0.001$).

Conclusions: ED of protein of extracted oilseeds estimated with the S. griseus protease test in the modification described here seems to come close to the in situ estimates. In contrast, ED of protein of legume grains, cereal grains and by-products from processing of cereal grains were mostly underestimated *in vitro*. It can be speculated that matrix effects could hinder the action of the protease at the target site. Pre- or co-inoculation with amylolytic and fibrolytic enzymes is probably required and should further be tested.

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99.

Opportunities and limitations for the standardization of the rumen simulation technique (RUSITEC) – a critical review

Möglichkeiten zur Standardisierung der ‚Rumen Simulation Technique' (RUSITEC) – eine Übersicht

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The increased public awareness of animal welfare and the aim of animal nutrition research to decrease the number of expensive and time consuming experiments with cannulated animals illustrate the need for further development and standardization of *in vitro* methods. The rumen simulation technique (RUSITEC) (1) allows maintenance of a stable ruminal fermentation for a longer period, the ability to investigate a large number of treatments in a short time period, to test high, in some cases potentially toxic levels of feed additives and to lower experimental costs (1, 2).
Methods: The aim of this review was to collect and compare technical and methodical variations of RUSITEC applications. It includes information from 93 studies published between 1977 and 2019 covering 111 individual technical variants. The focus was on the technical design and procedure of the experiments, including donor animal species, their feeding and the removal time of inoculum. The types and characteristics of equipment (e. g., engines, buffer pumps, vessels and buffers, incubation bags, incubation time and the duration of experimental runs) were compared.
Results: The collection process of ruminal fluid varied greatly between studies, with regard to technical details (e.g. collection time, handling of rumen fluid before incubation) as well as feeding of the donor animals.The pore size of polyester or nylon incubation bags ranged from 40 µm to 1000 µm. Especially, technical measures such as volume of vessels (from 0.5 L to 1.4 L), incubation time of feed bags (from 24 h to 72 h), particle size of incubated feedstuffs as effected by grinding or cutting and buffer composition showed a wide range.
Conclusion: The variability in the design and technical layout of the RUSITEC hampers direct comparisons among studies. The results of this review further support the need to standardizing the RUSITEC across laboratories, even if it is not feasible to standardize the different donor animal species. Currently, there are no uniform guidelines for the application of RUSITEC, unlike in other *in vitro* systems, e.g., the Hohenheim gas test. In summary, standardization defined in guidelines can reduce the variability in the design and application of the RUSITEC system and make results more comparable.

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100.

Effect of hay quality with or without concentrate supplementation on ruminal dynamic pH changes from birth to weaning in rearing calves

Einfluss der Heuqualität mit oder ohne Kraftfutterzusatz auf die pH-Wert-Änderungen des Pansens von der Geburt bis zum Absetzen von Aufzuchtkälbern

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An early maturation of the rumen is of high importance for rearing calves. Yet, feeding concentrate-rich starter to support the solid feed intake might induce ruminal acidosis in young calves. The aim of this study was to evaluate the influence of the composition of calf starter feed (only forage or mixture of forage and concentrate) and hay quality on ruminal pH of rearing calves.

Methods: In this study, 40 Holstein-Friesian calves (20 males and 20 females) were randomly grouped in four different dietary treatments based on the birth weight (42.89±6.19 kg). The calves (n=10 per group) were allocated into following starter feed groups: 100% high quality hay (HQH); 100% middle quality hay (MQH); 30% high quality hay + 70% concentrate (HQH+C) and 30% middle quality hay + 70% concentrate (MQH+C). Hay and concentrate samples were taken weekly for dry matter determination and every four weeks a pooled sample was taken and chemically analyzed. The hays differed in several nutrients including the sugar content (20.3% for HQH and 14.1% for MQH; dry matter basis) and neutral detergent fiber (45,7% for HQH and 51,6% for MQH; dry matter basis). The concentrate contained wheat (36%), barley (35%), soybean meal (17%), linseed meal (10%), and mineral feed (2%). All calves received the same amount of milk and had ad libitum access to starter feed and water. Samples of rumen fluid were taken using a stomach tube on days 7, 14, 21, 35, 49, 63, 77, 91 and 98 of life (sampling was approved by the Animal Experiment Authority). The pH was immediately measured using a pH meter (pH7+DHS, XS Instruments, Italy). Data were analyzed with SAS by one-way ANOVA using a model for repeated measures of samples taken from the same animal at different days. The significance level was set at 0.05.

Results: Solid feed intake of calves was very low in the first month of life. Calves started noteworthy intake of solid feed at week 4 to 5 and increased steadily, reaching up to 2 kg dry matter intake at weaning (12 weeks of life). At the end of the experiment (14 weeks of life), the calves had eaten up to 3 kg dry matter. The groups HQH, HQH+C and MQH+C had nearly the same dry matter intake and differed from MQH-group which consumed 1 kg less dry matter. Results showed a time effect ($P<0.05$) on ruminal pH course. Calves had a relatively high pH on day 7 (on average 6.40), which lowered on day 35 (6.26). Then the ruminal pH increased to the maximum of 6.79 by day 98. The ANOVA among groups revealed that feeding the MQH diet increased ($P<0.05$) the ruminal pH (6.68) compared with other groups such as MQH+C (6.44) and HQH+C (6.31), with the latter groups being not different ($P>0.05$). The HQH group had a pH value of 6.54. Male and female calves had nearly the same pH values, there were no significant differences ($P>0.05$).

Conclusion: The first results of this research indicate ruminal fermentation even before they consume noteworthy amounts of solid feed. To support this conclusion, we will further investigate the short chain fatty acids in the rumen fluid. Solid feed intake increased rumen pH, which was more emphasized in the group of middle quality hay, whereas the concentrate lowered the pH regardless of hay quality.

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101.

Changes in ruminal SCFA and ammonia concentrations in response to a daily increment of concentrate in the diet fed to dairy cows

Einfluss einer graduellen Erhöhung der Kraftfuttermenge in der Ration auf die Pansenfermentation bei Kühen

*Ricci S., Rivera-Chacon R., Castillo-Lopez E., Petri R.M., Reisinger N., Zebeli Q. – Vienna / Sherbrooke / Tulln a. d. Donau

Feeding grain-rich diets impairs the activity of rumen microbiota in cattle causing dysbiosis and dysfermentation (e.g. Subacute Ruminal Acidosis). A gradual adaptation to a high starch diet is expected to counteract dysfermentation, but there is limited information about the adaptation process of rumen microbes to gradual increase of concentrate intake. The aim of this study was to investigate the activity of the ruminal microbiota to daily dietary concentrate increments by evaluating the products of their metabolic activity (short chain fatty acids and ammonia) and ruminal pH.

Methods: Nine rumen cannulated dry Holstein cows were gradually shifted from a mainly forage diet to a high concentrate diet (26.3% grass silage, 8.7% corn silage and 65% concentrate on DM basis; 30.9% NDF, 28.5% starch) using a step-wise adaptation. Concentrates were increased by 10% daily in the total mixed ration, starting with 10% on day 1, to reach 60% on day 6 and 65% on day 7. Samples of ruminal fluid were collected every day for each of the 7 days at four hours after morning feeding. Ruminal pH was measured every 15 minutes with in-dwelling data loggers (Dascor Inc., Escondido, CA). Starch intake was calculated based on dry matter intake (DMI), which was recorded individually for each cow (Insentec B.V., Marknesse, The Netherlands). Short chain fatty acid (SCFA) composition was measured using gas chromatography, while ammonia was measured using the indophenol reaction assay. Data were analyzed using the Proc Mixed procedure of SAS with day as fixed effect and cow as random effect, and measurements on the same cow as repeated measures.

Results: The DMI increased between day 4 (11.7 ± 0.63 kg) and 5 (13.3 ± 0.63 kg) ($P < 0.01$), while starch intake increased every day for the first 6 days ($P < 0.01$), with the highest increments from day 2 (1.3 ± 0.13 kg) to 3 (1.8 ± 0.13 kg) and from day 4 (2.2 ± 0.13 kg) to 5 (3.0 ± 0.13 kg). Mean ruminal pH tended to decrease with the progressive inclusion of concentrate, from daily mean values of 6.5 ± 0.04 on the first day, to 6.0 ± 0.05 on the last ($P < 0.01$). Total SCFA increased on day 2 compared to day 1 ($P < 0.01$), and peaked on day 5, being greater than day 4 ($P < 0.01$). The concentration of SCFA decreased in the last two days when the concentrate level was 60 and 65%, respectively ($P = 0.05$). Acetate concentration decreased dramatically from day 5 ($P < 0.01$), while the release of propionate increased only on the last day ($P < 0.01$). Acetate to propionate ratio (A:P) decreased from day 1 to day 7 ($P < 0.01$). Butyrate production tended to decrease on day 3 ($P = 0.09$), and augmented on days 5 and 6 ($P < 0.01$). Total ammonia decreased on day 3 ($P = 0.01$). Values increased again to reach 27.02 ± 2.409 mM on day 5, but dropped on day 6 ($P = 0.05$) and day 7 ($P < 0.01$).

Conclusion: Gradual increase of dietary starch content at the expense of fiber caused changes in the products of microbial metabolism. The decreased concentration of SCFA in the last two days, accompanied with the increase of propionate and butyrate as well as the drops of pH and ammonia concentration suggest fermentative shifts towards subacute acidotic conditions already at the level of 60% concentrate in the diet. Further research focusing on the microbiome composition will help understanding the dynamics of the adaptive microbial process in order to minimize the risk of dysbiosis and increase rumen health.

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102.

Investigations on the congener-specific transfer of non-dioxin-like polychlorinated biphenyls from feed into chicken meat

Untersuchungen zum Kongeneren-spezifischen Transfer von nicht-dioxinähnlichen polychlorierten Biphenylen aus dem Futter ins Hähnchenfleisch

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants that accumulate in tissues of humans and animals. An important human exposure route are foods of animal origin. However, there is currently limited data available on the transfer behavior of individual non-dioxin-like (ndl) PCB congeners from animal feed into food of animal origin. It was hypothesized that individual ndl-PCB congeners may vary in their bioaccumulative properties in edible tissue and thereby in feed to food transfer behavior. To fill this gap, a feeding study was conducted with fattening chickens receiving a diet with a known ndl-PCB content.

Methods: A total of48 freshly hatched birds were divided into six groups of eight animals each. Animals were fed diets with known PCB contents for a fattening period of 37 days before slaughter. The control group received a commercial compound feed over the entire period with a very low background ndl-PCB concentration (control feed with 0.21±0.08 µg/kg ndl-PCB 88 %DM). Five treatment groups (Groups 1-5) received a commercial diet that was contaminated with ndl-PCB-containing paint in the loading cells of a feed company (11.7±0.4 µg/kg ndl-PCBs 88% DM) for different subperiods of time: 16, 23, 28, 32, and 36 days for groups 1-5, respectively. This feeding scenario reflected 20, 40, 60, 80 and 100% of total feed consumption of approximately 3500 g feed/animal during the fattening period. At the end of each subperiod, three animals per group were slaughtered to determine the congener-specific ndl-PCB content in liver and pectoral muscle. All remaining animals of each group were fed the control feed and kept until slaughter on day 37. The ndl-PCB concentration was analyzed by gas chromatography and high-resolution mass spectrometry and expressed either as µg per kg (88% DM) in feed or ng/g fat in animal tissue. To elucidate the congener-specific transfer properties of ndl-PCBs, physiologically based toxicokinetic (PBTK) models were derived from the experimental data and the physiology of fattening chickens.

Results: Although the maximum levels for feed materials of plant origin of 10 µg ndl-PCB/kg (88% DM) (sum of the six indicator congeners, (1)) were only slightly exceeded in the contaminated diet, already after 17 days of consumption of contaminated feed, the concentrations of ndl-PCBs in the meat of birds in group 1 (87.73 ng ndl-PCB/g fat) were more than twice as high as compared with the current maximum level (40 ng ndl-PCB/g fat (2)). Accumulation in meat quickly approached a stationary state in a fat basis, as evidenced by the scarce difference in the ndl-PCB content between group 3 (28 exposure days, 92.27 ng ndl-PCB/g fat) and group 5 (36 exposure days, 92.56 ng ndl-PCB/g fat). The modelling demonstrated that ndl-PCBs can be subdivided into two groups of congeners with similar kinetic properties: PCB 52 and PCB 101 as fast-eliminated congeners and PCB 28, PCB 138, PCB 153 and PCB 180 as slowly-eliminated congeners. Using the kinetic properties of individual ndl-PCBs, a backward modelling was conducted to predict the highest ndl-PCB concentration in feed that still allows for ndl-PCB levels in chicken meat below the maximum level. According to this modelling, a feed level of <4.4 µg ndl-PCB/kg feed (88% DM) would be required to ensure that the maximum permitted in chicken meat is not exceeded.

Conclusions: The present study evaluates the transfer of individual ndl-PCB congeners from feed into food-relevant tissues of fattening chickens and was used to derive a PBTK model. The model may help to understand congener-specific transfer and depuration scenarios in feed contamination events as a decision-support tool.

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103.

Ex-vivo gas- and organic acid production when incubating fiber products with colon inoculum from weaned piglets

Ex-vivo-Gasbildung und Produktion organischer Säuren bei Inkubation von Faserprodukten mit Dickdarminokulum von entwöhnten Ferkeln

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Fiber is a natural component in diets for all animals, although it has not historically been attributed any nutritional value. This perception is changing and especially towards specialty fibers due to beneficial stimulation of the gut systems through fermentation of non-starch polysaccharides (NSP) to organic acids and other metabolites. We hypothesized that different fiber types incubated with colon inoculum from weaning piglets affect concentration of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA). **Methods:** Two fiber sources A) a specialty soy fiber product (enzyme-treated) and B) a lignocellulose fiber product (mechanically processed) were incubated with digesta from 4 weaning piglets (15 kg). Samples were analyzed for contents of lignin (1), soluble NSP (sNSP) and insoluble NSP (iNSP) (2). Digesta from caecum and proximal colon were pooled 1:1, centrifuged and pasteurized to prepare a growth substrate, represent control. Fresh inoculum used to incubate test products were sampled from mid-colon digesta. A total volume of 10 ml was used in each simulation bottle: including substrate, fiber product (10 mg/ml) and inoculum (5%), blended under anaerobic conditions. 4 replicates were made of each fiber product, 4 replicates of control (no fiber added). The bottles were incubated at 37°C for 12 hours. Gas production was recorded every third hour. SCFA and BCFA were analyzed by gas chromatography after 12 hours incubation. Data were analyzed by Tukey's HSD test to separate means after ANOVA analysis. Differences were considered significant at $p<0.05$. **Results:** The fiber analyses showed that the specialty soy fiber product contained 17% sNSP, 50% iNSP and 2% lignin. The lignocellulose product contained 2% sNSP, 57% iNSP and 24% lignin. The specialty soy fiber product increased gas production throughout the incubation period compared to the control and the lignocellulose fiber product. Further, the total production of organic acids increased by 28% compared to control ($p<0.05$) and by 26% compared to the lignocellulose fiber product ($p<0.05$). No lactic acid was detected in the samples. Acetic, propionic and butyric acid concentrations increased by 33, 29 and 23%, respectively for specialty soy fiber product compared to the control ($p<0.05$). The lignocellulose fiber product resulted in same production of organic acids as control. Specialty soy fiber product decreased numerically the total BCFA concentration (1.30% of total SCFA) compared to control (1.70% of total SCFA) and the lignocellulose product (1.60% of total SCFA). **Conclusions:** Significant increase in butyric acid and decreased protein fermentation in colon indicates that weaned piglets can benefit from specialty soy fiber products and that competitive exclusion can be used strategically to stimulate lower gut health and function. The lignocellulose fiber product did not affect colon fermentation.

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Workshop

Reduce methane losses in cattle through feeding and breeding?

Principles of ruminal methane production, its implication to the greenhouse effect and natural mitigation limits

Grundlagen der ruminalen Methanproduktion, deren Bedeutung für den Treibhausgaseffekt und natürliche Grenzen der Methanreduktion

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Introduction

Ruminants, in particular cattle, have been identified recently as a "climate killer". To address this challenge, a multitude of scientists have been analysing the quantitative contribution of ruminants to global methane (CH_4) emissions, working on the identification of the rumen microbiome and developing CH_4 mitigation strategies. In particular, by use of high throughput metagenomics tools and innovative animal models, our knowledge and understanding of the rumen metabolism has enhanced substantially in recent years. This is a prerequisite for developing strategies for minimizing enteric CH_4 production by dietary, breeding, or management means. The interested reader is referred to some recent excellent reviews addressing various CH_4 mitigation strategies (Matthews et al., 2019; Lan and Yang, 2019; Beauchemin et al., 2020; Lassen and Dittford, 2020; Newbold and Ramos-Morales, 2000). Rather than attempting to summarize all these mitigation strategies, the aim of the present paper is to provide a brief overview about general principles of ruminal CH_4 production, its implication to the greenhouse effect and to the host, and to stress the biological boundaries in mitigating CH_4 emissions from ruminants.

Enteric CH_4 emissions and their contribution to the greenhouse effect

Atmospheric CH_4 is a sensitive indicator of climate change and an important factor contributing to the greenhouse effect. The greenhouse effect is the ability of atmospheric gases and the earth's surface to absorb solar infrared radiation. As a result, both the atmosphere and the earth's surface are heated and infrared radiation is re-emitted at a lower frequency (Moss et al., 2000). Due to its high absorptive power and cumulative radiative forcing (usually expressed in watts per square meter), methane's global warming potential is approximately 28-times higher than for CO_2 over a period of 100 years (GWP_{100} = 28) and 84 times higher for a horizon of 20 years (GWP_{20} = 84) (Myhre et al. 2013). While the atmospheric lifetime of CO_2 is 50 to 200 years, it is much shorter for CH_4 amounting to only 8-10 years (IPCC, 2019; Moss et al., 2000). The reaction with hydroxyl radicals produced from the photodissociation of ozone or water vapour in the troposphere is considered the major sink for CH_4 yielding CO_2 (Moss et al., 2000).

The mean CH_4 concentration in the earth's atmosphere present-day is 1.77 ppm, which is 2.5 times higher than observed for the period of 1000–1750, and higher than ever as observed in the ice-core record over the past 800,000 years (Loulergue et al., 2008). About 58% of the globally emitted CH_4 is of anthropogenic origin, but also wetlands (30%), oceans, lakes and rivers (7%) as well as termites, wildfires and increasingly thawing permafrost (5%) contribute to CH_4 emission (Knapp et al., 2014). Anthropogenic CH_4 sources include fossil fuel and biogenic sources but the latter make up a larger proportion of emissions than they did before 2000 (IPCC, 2019). Global CH_4 emissions from the agriculture, forestry and other land use sectors amount to 162 ± 49 Mt per year representing 4.5 ± 1.4 Gt carbon dioxide equivalents (CO_{2eq}) and 23% of total net anthropogenic emissions between 2007 and 2016 (IPCC, 2019). The livestock sector is the largest contributor of anthropogenic CH_4 emissions representing 94 – 99 Mt of CH_4 per year during this period (FAOSTAT, 2017), which corresponds to 6-7% of the global anthropogenic greenhouse emissions (Knapp et al., 2014). Another 15% CO_{2eq} are emitted due to land use changes associated with ruminant livestock farming (Knapp et al., 2014).

Besides minor CH_4 emissions from faecal and slurry management, the vast majority of the annual CH_4 emission rate originates from enteric fermentation. In 2018, most ruminant CH_4 emissions originated from non-dairy cattle (54 Mt), dairy cattle (18 Mt), buffaloes (11 Mt), sheep (6.7 Mt) and goats (5 Mt) (FAOSTAT, 2017). Conclusively, ruminant livestock certainly have the largest CH_4 mitigation potential, but the potential for reducing total net greenhouse gas emissions is much greater for other sectors like industry (29%), energy supply and transportation (28%), residential, commercial and public services, and waste management (21%) (Knapp et al. 2014). Yet, a global target for reducing emissions from agriculture of ~1 Gt CO_{2eq} per year by 2030 has been identified to limit warming in 2100 to 2°C above pre-industrial levels, and the reduction of CH_4 emissions is key in this strategy (Wollenberg et al., 2016). This ambitious aim is confronted with biological boundaries of rumen fermentation as optimizing formu-

lation of rations in practical use or feeding supplements may reduce CH_4 emissions realistically by only 10 – 30% (Knapp et al., 2014).

When systemic boundaries are widened from animal to farm, ruminant livestock farming may not just be a net CH_4 emitter but also serve as carbon sink. Depending on the stocking rate and intensity, grazing increases the net storage of C in grassland soils, despite higher net CH_4 emissions and removed biomass (Gomez-Casanovas et al., 2018; Bork et al., 2019).

Rumen microbiome and fermentation

The digestive tract of ruminants has evolved to ferment and digest large amounts of plant biomass. The rumen is one of three proventriculi and the largest compartment of their digestive tract. As the primary fermentation chamber, the ingested feed, water and swallowed saliva is incubated with a highly diverse microbial community consisting of bacteria, archaea, protozoa, fungi, and some non-cellular life such as phages, and occasionally helminths and transitional organisms. An oxygen-free environment and a temperature between 37 and 42°C supports continuous diet fermentation and microbial growth. Moreover, the transport of small molecules, i.e. CO_2, ammonia and urea from the circulation to the luminal site and vice versa, ensures buffering of the ruminal pH between 6 and 7 and provision of important nutrients for microbial growth. Microbes can be found in three rumen compartments, namely in the solid (adherent) phase (70%), the liquid phase (25%), and in and attached to the rumen epithelium (epimural; 5%) (Matthews et al., 2019). One mL of rumen fluid may contain 150 billion microorganisms, among them 10^{10} to 10^{11} bacteria, 10^4 to 10^6 ciliate protozoa, 10^6 to 10^8 archaea, and 10^3 to 10^6 fungi. It has been suggested that the liquid-phase microbiome is more diverse than the solid phase (Jewell et al., 2015) and contains more than 500 cultured species (Newbold and Ramos-Morales, 2000). By contrast, more than 4900 novel microbial genomes from the rumen of cattle have been identified so far while major metabolic pathways have been assembled for only 75% of these genomes (Newbold and Ramos-Morales, 2000). The core community of bacteria, which is the common microbiome identified from various animal species across numerous countries all over the world, consists of 30 bacterial taxa including *Prevotella, Butyrivibrio*, and *Ruminococcus*, as well as unclassified Lachnospiraceae, Ruminococcaceae, Bacteroidales, and Clostridiales (Henderson et al., 2015). Rumen bacteria can be classified based on their substrate preference such as fibrolytic (cellulolytic), amylolytic, proteolytic (Henderson et al., 2015), lipolytic, acetogenic, ureolytic or tanninolytic (Sirohi et al., 2012). When cellulose and hemicellulose, major components of plant fibre, are ingested, primary fermenters such as *Ruminococcus* and *Fibrobacter* species degrade these polysaccharides yielding hexoses and pentoses as well as succinic, formic or acetic acid. The primary fermenter for starch is the amyolytic bacterium *Streptococcus bovis* that produces lactic acid, whereas pectin is converted by Lachnospiraceae yielding oligogalacturonides (Metthews et al., 2019). Intermediary products from primary fermenters such as glucose, succinic and lactic acid or ethanol are either further processed by the same species or utilized by other bacteria, so-called secondary fermenters. End products of primary and secondary fermenters are predominantly acetate, propionate, butyrate, CO_2 and H_2 with the former three short-chain fatty acids (SCFA) serving as major energy sources for the ruminant host. The dietary constitutes influence the formation of the relative proportions of these end products in parallel with the microbial community.

The glycolysis of poly- and monosaccharides towards SCFA and CO_2 is an oxidation reaction executed under anaerobic conditions. This is achieved by the production of hydrogen equivalents (NADH) generated in the conversion from glucose to pyruvate (Embden–Meyerhof–Parnas pathway). The oxidation of pyruvate towards acetate and CO_2 yields further NADH, whereas the reduction of pyruvate to propionate or butyrate consumes NADH. The re-oxidation of NADH in the presence of protons is facilitated by hydrogenases yielding H_2. Moreover, H_2 is also the major fermentation end product of protozoa and fungi. Nevertheless, H_2 does not accumulate to high concentrations in the rumen as it is utilized by other microbes (interspecies hydrogen transfer) to reduce for example nitrate to ammonium, sulphate to H_2S, fumarate to succinate, or CO_2 to acetate, but the largest sink of hydrogen utilisation is the reduction of CO_2 to CH_4 (Moss et al., 2000). Hydrogen, which is not used by microbes, is eructated by dairy cows at a rate of 0.5 and 3 L/h, which is 8 to 80-times lower than the accompanied CH_4 eructation rate (Olijhoek et al., 2016). Elimination of hydrogen via microbial oxidation processes or eructation is of high importance because hydrogen inhibits microbial growth and fibre fermentation (ROOKE et al. 2014). Furthermore, a very low hydrogen partial pressure of 1-10 Pa is necessary to allow for the re-oxidation of NADH (ELLIS et al. 2008).

Methanogenic archaea and methanogenesis

The largest sink for hydrogen (78%) in the rumen are hydrogenotrophic methanogens and most of them utilize CO_2 as an electron acceptor as follows: $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$. The most abundant hydrogenotrophs are *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium*, which make up 74% of the rumen core microbiome, while *Methanosphaera*, *Methanimicrococcus* and *Methanobacterium* are minor abundant hydrogenotrophs (Seshadri et al., 2018). Other methanogens (22%) transfer the hydrogen to methyl- and dimethylamines (methylotrophs): $CH_3NH_2 + H_2 \rightarrow CH_4 + NH_3$. Those methylotrophs include *Methanosarcinales*, *Methanosphaera*, and *Methanomassiliicoccaceae* genera (Seshadri et al., 2018).

Rumen methanogens belong to the domain archaea, which are strictly anaerobic, however, not all of them produce CH_4 via hydrogen transfer. A minor portion of methanogens reduce acetate (acetoclastic methanogenesis) as such: $CH_3COOH \rightarrow CH_4 + CO_2$. Moreover, *Methanosphera stadtmanae* reduces methanol as shown in the following reactions: $4\ CH_3OH \rightarrow 3\ CH_4 + CO_2 + 2\ H_2O$ (FERRY 1992) or $2\ CH_3OH + CH_3CH_2OH \rightarrow 2\ CH_4 + H_2O + CH_3COOH$ (HOEDT et al. 2016). Thus, ruminal CH_4 production does not exclusively depend on hydrogen supply. This is, among others, one reason why the attempt of directing hydrogen towards acceptors other than CO_2, i.e. nitrate (Olijhoek et al., 2016), may reduce but not fully abolish CH_4 production, although the hydrogen transfer to nitrate is thermodynamically more favourable than to CO_2. On the other hand, diet supplementation with nitrate may lead to methemoglobinemia, compromising animal health.

Methanogenesis is essential for all ruminal archaea as it is their sole pathway to generate energy from anaerobic respiration. Archaea numbers reach as much as $10^7 – 10^9$ cells/ml rumen content, amounting to 0.3 to 3% of the rumen microbiome (Newbold and Ramos-Morales, 2000). However, archaea are more abundant in the solid phase, suggesting that liquid-phase rumen samples may not give a full picture of the relationship between the rumen microbiota and CH_4 emissions (Bowen et al., 2018).

Methanogenesis is under enzymatic control and may be confined when the hydrogen partial pressure is too high or the enzyme methyl-coenzyme M reductase (MCR) is inhibited, i.e. by 3-nitroxypropanol (Duin et al., 2016). Accordingly, there is a direct relationship between CH_4 production and *mcrA* DNA abundance (Aguinaga Casañas et al., 2015), however, other studies showed that the archaeal enzyme activity is more related to CH_4 production than archaeal abundance (Shi et al., 2015).

As CH_4 formation consumes hydrogen whereas the formation of acetate produces hydrogen, a strong positive correlation between CH_4 production and ruminal acetate concentrations can be found. Contrary, propionate that is produced from pyruvate consuming hydrogen is negatively correlated with CH_4 production (Aguinaga Casañas et al., 2015). However, dietary support of the propionate producing pathway, i.e. increasing the level of starch in the ration, may induce rumen acidosis, again compromising animal health.

Meaning of CH_4 for the host

The energetic loss due to CH_4 emissions represents 2 to 12% (Johnson and Johnson, 1995) of the gross energy intake of dairy cows. Theoretically, inhibition of CH_4 formation increases the efficiency of conversion of digestible energy to metabolizable energy by decreasing CH_4 energy losses (Johnson and Johnson, 1995). This is, however, highly speculative as ruminal microbes improving feed digestibility are not associated with CH_4 emissions (Roehe et al., 2016). In addition, ruminal CH_4 has an important role in the regulation of rumen motility. Dittmann, et al. (2016) demonstrated that low levels of ruminal CH_4 were associated with less rumen contractions, higher mean retention time of the digesta, greater apparent digestibility of crude protein and neutral detergent fibre, and longer rumination times. From these observations it seems highly likely that the post-absorptive metabolism is affected similarly, but so far no such study has been conducted.

Conclusions

Due to its quantitative contribution to global greenhouse gas emissions (less than 10% of the total), ruminants are not climate killers. Yet, livestock is a contributor to these emissions and the latest increase in net enteric CH_4 production is alarming. Due to biological and thermodynamic constraints, a significant reduction of CH_4 emissions from ruminants, i.e. by more than 30%, cannot be achieved by dietary means only as this bears the risk of compromising animal health when dietary supplements are overdosed. Therefore, a combination of optimizing diet to maintain animal health while reducing CH_4 emissions, optimizing pasture grazing and stocking rate to sequester carbon in soil, and reducing animal numbers appear to be more effective. The latter could be achieved easily by avoiding waste of foods.

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Possibility of using mid-infrared (MIR) spectra of milk to estimate methane emissions from cows

Möglichkeit der Nutzung von mittleren Infrarotspektren (MIR) der Milch zur Abschätzung der Methanemission von Kühen

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Introduction

Availability of methods to estimate easily and cost-efficiently individual methane emissions (CH_4) for a large number of animals is the major bottleneck in the development of strategies to reduce CH_4 losses through feeding and breeding. Some advanced techniques have been developed to measure CH_4 in a research setting (e.g., feeding trials), but they are not well adapted (e.g., expensive, difficult to manage on farm) for large-scale routine on-farm recording. Other researchers have explored different indicators or indirect traits, often called proxies, to estimate CH_4 emissions. One technology that has appeared as promising, inexpensive, and amenable to being used individually on a large scale, still staying sufficiently accurate to distinguish low from high emitters, is the use of mid-infrared (MIR) spectrometry. The use of this technology on milk samples generates spectral 'fingerprints' of fine milk composition. This review will (a) describe briefly current "direct" methodologies to measure systematically CH_4 in lactating dairy cows, (b) extend to milk-based CH_4 proxies, (c) provide an overview how strategies to use MIR spectra in milk to estimate CH_4 emissions were developed, and (d) do a critical evaluation of the associated scientific, but also technical (e.g., standardization of MIR spectra) and organizational (e.g., consortium building) issues.

Current 'direct' methodologies to measure CH_4 in lactating dairy cows

There is still a vivid discussion which CH_4 phenotype is best suited (i.e., CH_4 production (MeP) in liters or grams per day; CH_4 intensity (MeI) defined as liters or grams of CH_4 per kilogram of milk, CH_4 yield (MeY) defined as liters or grams of CH_4 per kilogram of dry matter intake (DMI); residual CH_4 production (RMP) defined as observed minus predicted CH_4 production). If all these units may be suitable for specific and different purposes, they all need the knowledge of basic CH_4 emissions. Therefore, many different direct methods were developed to measure CH_4 emissions, also feeding on advances in technologies. Without pretending to be completely exhaustive, these methods include measures in respiration chambers (RC), measures using breath sampling during milking (e.g., sniffers in AMS) or feeding (e.g., GreenFeed system), measures obtained using portable equipment (laser detectors); or those relying on the tracer technique based on Sulphur hexafluoride (SF_6). A recent review by Garnsworthy et al. (1) gives relevant details on the available methods and compares them according to their main feature of methods for measuring methane output by individual animals. These authors compared RC, SF_6 technique, breath sampling during milking and feeding, GreenFeed and laser methane detectors according to diverse criteria as purchase costs, running costs, required labor, repeatability, behavior alteration and throughput. Based on the experiences of a large group of scientists involved in the EU COST METHAGENE project, they concluded that these methods are never associating at the same time low purchase, running and labor costs with high repeatability, no behavioral alteration and high throughput. When integrating all these criteria, the two extreme methods are RC and (portable) laser methane detectors ('laser guns'). Respiration chambers are considered being the 'gold standard'. They also detect all the CH_4 emitted by the animal, but they influence massively animal behavior, and are unsuitable for large-scale measurements of methane emissions. Laser guns are portable, therefore could be easily transported from farm to farm, but these tools are difficult to handle, therefore a skilled labor force is necessary. The SF_6 technique is also very labor-intensive and our experience shows that its use outside of research herds with animals not used to be handled constantly is very tricky. It has to be stressed here that these methods can be grouped in those doing continuous measurement (e.g., RC, SF_6) and others that only do discontinuous sampling. The fact that discontinuous measurements are only integrated in a second step into continuous (e.g., daily) measures should not be forgotten when doing comparisons. Another point not mentioned by Garnsworthy et al. (1) is that methods have to generate repeatable measurements (i.e., same methodology) also for different experiments at the same place but also at different places. This point will be very important for the development of datasets with sufficient reference records necessary to generate reliable and robust CH_4 prediction equations (2). Here the use of a standardized method as the GreenFeed apparatus could obviously be the preferred way forward. This explains also, why some breeding organizations have been heavily investing in this approach despite the high price of one apparatus (several 10 k€).

Giving that one free access GreenFeed can service maximum 50 cows, for following 1000 cows, we will need already 20 apparatuses.
Similarities of rankings and predicted values among methods are another source of constant debate. What is important is that Pearson correlations of different alternative methods to RC are high but still far from unity (1) with values between 0.72 and 0.89. However, when using Lin's concordance correlation coefficient (CCC), which is the expected value of their squared differences, between different methods these values decrease, often dramatically (1), showing values between 0.30 and 0.88. The reason for this is that even if the Pearson correlations are high, the values of CH_4 found by each method follow different scales illustrating bias (mean differences) and slope (variance differences) when compared to chamber results. Many biological, practical and technical reasons explain that all methods can only measure CH_4 with some level of error, or more precisely, each method measures 'a' CH_4. Therefore, this opens the debate if there is any 'true value' as even the 'gold standard' (i.e., RC) has some serious flaws (e.g., artificial environment, behavioral alterations) especially when used with high-producing, potentially very sensitive, dairy cows.

Milk-based CH_4 proxies

Before establishing the current status of research on milk-based CH_4 proxies, we want to stress that many other strategies were proposed to get these proxies based on different more or less available traits (e.g., DMI) (3). As a matter of fact, if measuring emitted methane on lactating cows is a challenge, it is an opportunity that we have potentially access to their status on methanogenesis for each milking. Even if milk yield and major milk components were not considered good predictors of CH_4 emissions (MeP) (3), the use of other, finer, components was recognized as being promising. It is generally accepted that fatty acids (FA) are the best candidates here. The rationale is that there is a direct link to the fermentation process of dietary carbohydrates in the rumen that leads also to the production of H_2. It also general knowledge (3) that the synthesis of acetate and butyrate produces H_2 that is then converted to CH_4, while the propionate synthesis consumes H_2. Many different prediction equations have been developed to describe relationships between milk FA (measured using gas chromatography) and enteric CH_4 emissions and to allow predictions based on milk composition. Negussie et al. (3) reported that there were equations available from at least 9 studies, most of them were reviewed by van Gastelen and Dijkstra (4). The prediction equations developed were very different for the FA considered and showed wide ranges of accuracies. These authors and others attributed these facts to differences in reference methods but also to CH_4 trait definition (MeP, MeY, MeI, RMP). Moreover, these equations were nearly all based on specific experiences, therefore, one might speculate that each represented a specific situation. Also, at least one study (5) illustrated that links between CH_4 and milk FA are not constant throughout the lactation. This finding has two consequences. First, it could add to the understanding of the large diversity of results in literature. Moreover, it shows the interest to include also lactation stage inside CH_4 prediction equations. The influence of this factor was already illustrated in a study (6) on the variation of individual CH_4 measurements made on farm during milking.

Strategies to use mid-infrared (MIR) spectra of milk to estimate CH_4 emissions

As explained in the previous paragraph, even if milk based CH_4 proxies might look attractive, there are several issues that block their large scale use. First, the FA that are used in these equations were obtained using gas chromatography (GC). Gas chromatography on a large scale is very costly (around 100 €/analysis). The usefulness of GC based fine milk composition is therefore limited by its availability (7). Since many years, routine milk analysis relies on the analysis of milk using MIR spectrometry (7). This technology relies on the interaction of matter with infrared light, by absorbance or reflectance. A now well-established strategy (8) in milk analysis is the use of the Fourier-transform MIR technology therefore often called FT-MIR. In its practical use, we associate MIR spectra with reference data in a first step, generating prediction equations (9) that can then be used in the field to predict the traits of interest. This was successfully demonstrated to be feasible for many FA (10) and other traits of interest (e.g., 6, 7). As MIR data is generated in routine milk analysis, both for DHI but also for payment samples, if access to this data can be obtained what was until recently the major bottleneck, traits of interest (here FA) can be predicted and this, to a certain extent, even retrospectively. For this reason, a first strategy was to predict FA needed for existing CH_4 equations from MIR (MIR-FA) and to use them in the equations. Unfortunately, many of the equations rely on specific FA that are not well predicted using MIR; therefore, MIR-FA could not replace GC-FA.
An important issue, that will be explained in detail in the next paragraph, is the availability of reference data for the

development of CH_4 prediction equations. Due to the reduced availability of CH_4 reference data, hybrid methods were developed using GC-FA predicted CH_4 as references (dependent variable) and available MIR-FA as independent variables. In a second development step, researchers replaced MIR-FA data by using direct MIR spectra data (4, 11). This required a high level of synchronization between GC analysis and MIR data collection but avoided the necessity to have direct measurements. All these developments were obviously only very sub-optimal approaches because accumulating prediction errors of the references with the prediction errors in their prediction from MIR-FA (or MIR) is not necessarily a sound approach. Therefore, the idea appeared to avoid these cascading errors and to link directly CH_4 to MIR spectra as first tested and reported by Dehareng et al. (12).

Overcoming scientific, technical and organizational issues in the use of MIR data to predict CH_4

Developing methods, equations and strategies to predict CH_4 direct from MIR data requires the solving of many associated scientific, technical and organizational issues (2). The first issue with MIR spectral data is that the analysis of the same milk sample on different machines will generate different spectra. For the build-in estimation of major milk components, manufacturers deal with this issue internally but users are still required to run reference samples with known composition to certify fat and protein contents. This allows to do bias and slope corrections. Unfortunately, for novel traits, and especially for CH_4, we do not have this type of calibration samples with known values. An alternative approach was developed that is called 'standardization' in literature (13), doing bias and slope corrections on an elementary wavenumber level using reference samples. This approach was setup in a way that interpolations between spectrometer brands (and types) are also possible in a common range, as the range of covered wavenumbers and the specific numbers is not identical (14). The use of this strategy has two consequences. First, it allows that CH_4 records from different experiences can be combined across apparatuses used to get associated reference MIR data (14). The second is that the developed equations can be used on any device that is regularly standardized.

A second issue is that robust MIR calibration equations need the largest possible variability present in the reference data, both for the dependent variable (here CH_4) and for the independent variables (here mainly MIR spectral data, but also other factors used as lactation stage). Potential data sampling stratifications to cover also sub-populations is also advantageous. In order to achieve this goal, because of its rarity, CH_4 data needs to be sourced from a large variety of experiences reflecting expected field conditions where the developed equations will be used. Currently, a major challenge is to consider feeding additives or other means that disrupt natural methanogenesis. A better understanding is needed how these methods work and what their effects on fine milk composition are, before we can access how we could correct for their use.

The need for more, divers reference datasets, creates many different issues because this data has to be harmonized. If the 'standardization' procedure allows assembling MIR datasets, linked CH_4 reference data are still sparse. Also, many different CH_4 reference methods and implementations are used. Therefore, in the first stages of our research, we used SF_6 data that was considered to be based on a rather precise protocol, taken with limited disruptions for the animal. Sourcing on data from Wallonia (Belgium) and Ireland, first versions of the CH_4 equation was developed (12). During this process, it appeared that prediction of MeP is a better target variable than MeI or MeY. The importance of lactation stage (15) was also established. The research to test new and different explicative factors as milk yield, lactation rank or breed is currently ongoing (2) and some of the issues presented in this review are under scrutiny.

Already these first steps in the equation building illustrated strongly the impact of limited data. Therefore, an organizational requirement appeared very quickly to create a large consortium, allowing the creation of common models for MIR-based prediction of CH_4. In the MIR world, there is a tradition of using spectrometer supplier-provided equations (e.g., fat content), but we found inspiration in the near-infrared spectrometry (NIR, often called NIRS) world for an alternative approach. The existence of NIR forage and feed-testing consortia is well known in the animal feed industry. We are applying this strategy of "Open" Consortium Building (OCB) to the MIR world. The intention of OCB is not to create global research datasets shared by all, which requires extensive confidential agreements between all partners. The idea of OBC relies on a general framework, which defines roles as calibration building organizations (CBO) or consortium members (CM) providing data. These CM retain full ownership and subscribe only to a common goal, the improvement of CH_4 equations. Calibration building organizations will be the only organizations obtaining full access to the data, all CM retain full control over their data and can use it freely for any other purposes. They provide data to CBO only to advance the development of better and more robust

equations. Consortium members get access to the equations and can implement them allowing them access to MIR-based CH_4 predictions. They will also have access to all future updates when new equations become available due to new data or improved calibration methodologies. The main advantage of this strategy is that different CM have not to grant each other access to their respective data, which may become very difficult to negotiate, especially if some CM have competitive interest. In this alternative arrangement, CM will only grant access to CBO and this only for this specific purpose. This allows keeping the highest level of confidentiality and should allow industry-funded data to contribute as long as they accept the underlying concept of win-win. We were very successful using this strategy for the development of MIR-FA equations and currently we use it in the process of MIR prediction equation building for CH_4.

Current status of the development of MIR-based CH_4 predictions and possibility of their use

The development of MIR based CH_4 predictions has been well documented through a certain number of peer-reviewed publications between 2012 (12) and the moment of this communication (2). Basically, two reference CH_4 data sources have been used until now. Research using GreenFeed data has only started. First efforts used only SF_6 data (15) and RC data (16) separately. Latest results using and integrating SF_6 and RC were reported by Vanlierde et al. (2). Data from RC experiences came from CH, DE, DK, FR and UK. A first conclusion that can be drawn from these results is that the SF_6 based equation behave better when used to predict RC-CH_4 then the inverse where the RC equation was used to predict SF_6-CH_4 (Root Mean Square Error of Prediction RMSEP of 105 g/d vs. 140 g/d). This result and other observations made us belief that each RC dataset represents a specific experience leading to prediction equations that are not yet robust enough. As already stated before, this type of specificity makes the development of robust equations on RC data alone difficult. However, we also noticed that data from these two CH_4 methods (SF_6: n = 531 and RC: n = 576) are similar enough to be combined leading to a R_{2C} of 0.68, R_{2CV} of 0.64 and a CCC of 0.79. This combined model accounts for a maximum of spectral and CH_4 variability. Following the outline of Grelet et al. (9) this level of accuracy allows to compare groups and to discriminate high from low values. Currently, we are expecting several directions of developments. First, we are trying to assemble more reference data obtained with SF_6 or RC trying to cover variabilities not yet accessed. The OCB strategy would allow new CM to enter the calibration effort and all partners to benefit. Alternative reference methods are also currently explored. We have started to study the feasibility of GreenFeed-based equations and are proposing the same OCB strategy to new collaborators. The advantage of GreenFeed data is that it is theoretically highly harmonized and many research groups and industry players are installing this tool even in production farms. According to results reported recently (1) differences are expected, especially between results using GreenFeed and SF_6. One specific issue with GreenFeed data is that specific efforts are needed to synchronize CH_4 (in GreenFeed recorded over a longer period but integrated from discontinuous measurements) and specific MIR records (at each milking). Moreover, despite its harmonization, GreenFeed data might need some specific quality control measures that are not currently used in SF_6 and RC. Our experience is that data quality issues in reference data (e.g., taken under farm condition) can be a major obstacle for robust equations.

A second line of development is the addition of other factors responsible for the variation of CH_4. First we have noticed the importance to consider the lactation stage and it has been included (15) in order to make the prediction equation lactation stage-dependent. Based on the study of prediction residuals, a recent study (2) has demonstrated the importance of milk yield, parity and breed. Modification of prediction equations to include these effects showed moderate improvements with R_{2C} of 0.73, R_{2CV} of 0.68 and a CCC of 0.81. Alternative strategies are under investigation. The disadvantage of this type of adaptation is that it may complicate the use of the equations if this factor is not routinely recorded.

Another issue that is under constant scrutiny is the use of alternative calibration strategies. Many ideas exist here going from alternative sample selection approaches to the use of alternative machine-learning type methodologies (17) The improving of calibration strategies is clearly a topic that can be a seen as scientific extension of the OCB efforts.

Conclusion

The use of fine milk composition and especially of MIR spectra to predict CH_4 has at least two major advantages. First, it feeds on similar pathways like those generating CH_4 internally in the animal. It also does not try to capture emitted CH_4. A disadvantage is that feeding additive or other means that disrupt natural methanogenesis will pro-

bably also render MIR-based CH_4 prediction less reliable, at least at this stage. Second, MIR spectra are already obtained in routine, in a repeated and repeatable fashion, and on a large scale. Therefore, under the hypothesis that there is no systematic bias, repeated MIR-based CH_4 estimates should allow to give reliable rankings of animals for their CH_4 emissions over a longer period. Management and breeding of dairy cows can largely benefit from the large-scale generation of this kind of data. Current equations are only a snapshot of an ongoing effort. A collaborative approach is pursued the assembling of more and alternative reference data. Moreover, recent advances (e.g., machine learning) show the potential to improve used calibration methodologies.

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The role of genetics for methane emission in cattle

Rolle der Genetik für die Methanemission bei Rindern

*Swalve H. – Halle

Introduction

Methane emissions from the cattle sector have been held responsible for a critical role of farming with respect to climate change although they account for only a small fraction of total greenhouse gas emissions. Methane emissions are an inherent property of ruminants and hence the ultimate goal is not a reduction towards zero emissions but rather a significant reduction which is certainly desirable. In principle, methane emissions might be mitigated by different diets, management of the microbial population of the rumen, and by genetic selection. The latter approach requires that heritable traits can be defined and recorded which enable breeding for reduced emissions. The advantage of a mitigation strategy by genetic selection is that changes achieved are permanent and cumulative whereas other strategies depend on a continuous treatment or a continuing diet. For the entire dairy sector, Wall et al. (1) identified three approaches for genetic selection: (i) an increase in efficiency, (ii) improving of functional and health traits thus reducing the need for involuntary culling, and (iii) direct selection for decreased emissions. Approach (i) indeed has been applied by dairy cattle breeders around the world as milk yields have increased at a higher rate than feed intake and at the same time less cows have been needed for equal total production thus reducing the total amount of feed needed and emissions due to maintenance. In terms of efficiency per unit of milk produced, on the basis of gram of methane emissions per kg FPCM, methane emissions have decreased by 13 % in the period of 1990 to 2010 in the Dutch dairy sector (2). When improving functional traits and reducing the need for involuntary culling, less animals, young stock and milking cows are needed in the dairy sector. Following approach (ii), genetic selection for robustness and improved resistance to diseases has been put in place in many countries in recent years with advanced technologies for dairy cattle breeding. Direct selection for a decrease in methane emissions (iii) offers the most rapid way of a desired change. The prerequisite, however, is that a framework can be set up which enables genetic selection. In this presentation, different aspects for this framework are discussed.

Requirements for genetic selection

From the viewpoint of animal breeding, methane emissions are to be regarded as any other new trait which is considered to be included in genetic selection. Thus, knowledge on the following parameters is essential:

- Phenotypic variation (σ_P^2), the amount of variance on the observable, phenotypic scale
- Additive-genetic variation (σ_A^2), the amount of variance on the additive-genetic level, derived from statistical analyses of related and unrelated individuals
- Heritability (h^2), the ratio of (σ_A^2) / (σ_P^2) as an indicator of the fraction determined by genetic effects
- Phenotypic and genetic correlations with all other traits in the breeding goal and with other traits that could be of relevance
- The economic value of a trait, defined as the marginal profit achievable when the natural value of the trait is changed by one unit

As selection response, also denoted as genetic gain, is a purely multiplicative function of selection intensity, heritability and the phenotypic standard deviation (σ_P), it is worthwhile noting that a response to selection may be achieved even for cases with low heritability if a substantial variation is available. The knowledge of phenotypic and especially genetic correlations is essential when deriving a breeding goal as genetic correlations are decisive for possibilities of supporting genetic progress for a specific trait in the case of positive correlations or for detrimental effects when correlations are negative. The optimal balance for all traits in the breeding goal can be derived by applying the methodology of a selection index as this tool incorporates the knowledge on all parameters listed above with the target function of optimal overall genetic progress in terms of economic value. One important aspect of selection index methodology is that it is not advisable to include new traits consisting of ratios of traits that may already have entered the breeding goal. For methane emissions, a phenotype defined e.g. as methane emission in g per day divided by FPCM could have almost unpredictable consequences when applying a selection index as milk production is quite naturally already in the breeding goal.

For the case of methane emission, a reduction does not have an economic benefit for farmers at present. If emissions would be assigned a monetary value, e.g. in the form of taxes, this could change dramatically and, in this case, methane emissions could be included into a breeding goal with a negative weight. Another way for methane emissions to enter the selection index framework would be if recording on emissions would be of use as an auxiliary trait, e.g. for feed efficiency. This would require, however, that methane emission records could be collected in a precise manner and for a large number of animals.

The question on the precision and feasibility of collecting methane emission records is a general one, it is even more important when methane emissions would be a main trait within the breeding goal. The precision of measuring a phenotype affects all parameters listed above. It is especially important to note that also heritability, which is commonly regarded as a parameter purely following biological rules, may change substantially for different precision levels of recording.

Recording of methane emissions

For direct recording of methane emissions, a number of technologies exist as reviewed in (3). The 'Gold Standard' in use since many years are respiration chambers (RC) for open- or closed-circuit indirect calorimetry. Although measurements from respiration chambers are highly accurate for a given unit, measurements from different chambers at different sites may vary due to differences in air flow rate and actual measuring within the flow. However, still most studies attempt to validate alternative methods of recording against RC which is inherently difficult as animals cannot be placed in RC and measured under e.g. barn conditions at the same time. Apart from the high costs of purchasing and operating RC, a main problem with RC is that they severely affect the behavior of an animal which is put into this very artificial environment. Drastic changes in feed intake as well, for the case of lactating cows, in milk production, are often observed.

The sulphur hexafluoride (SF_6) tracer gas technique is based on placing a permeation tube with SF_6 inside the rumen, then collecting samples of breath from the nostrils of the animal and storing these samples in a canister mounted to the neck or back of the animal. The canister has to be emptied every day and from the concentration of SF_6 gas mixed with methane, emissions of methane can be quantified taking into account the pre-determined release of SF_6 from the tube. The method has been shown to be fairly accurate when compared to RC, a disadvantage being the requirement for highly skilled operators.

Most studies attempting to estimate genetic parameters as required for genetic selection have been using the 'sniffer method' for recording of methane emissions per animal. The sniffer method uses sampling tubes placed in robot milking units or feeding stations and connected to a gas analyzer. Thus, samples are taken not continuously but rather several times per day. Sampling time per measurement will be several minutes thus enabling to detect eructation peaks. Recording with sniffers is relatively inexpensive, the technology can be installed in commercial farms and is not invasive as animals are not aware of the measurements being taken. The sniffer method is suitable for high throughput as needed for genetic studies.

The GreenFeed system (C-Lock Inc., Rapid City, SD, USA) is a more sophisticated sniffer system capable of measuring animals at a feeding station. It also controls airflow and hence results are fairly accurate and apart from concentrations of methane it also provides a phenotype expressed as methane in g/day. Costs and throughput are directly related to the number of GreenFeed stations that are purchased and operated.

The laser methane detector (LMD) is a small, hand-held device originally developed to detect gas leaks in mining. The measurement is in ppm units per m of distance between operator and the animal's nostrils. Hence a conversion from concentration to mass of emitted methane is necessary. The main advantage of the LMD is its flexibility, it can easily be carried from animal to animal and from farm to farm. For improved accuracy, a protocol is advisable consisting of repeated measurements covering several minutes of measuring to capture eructation events. Our group now has a > 5-year experience working with three LMD on commercial farms and at experimental stations. Our protocol consists of measuring each animal for five minutes for one profile, at the same time of the day, and at the same activity, for three consecutive days. Results from the profiles can be analyzed as averaged values or as repeated records later. The full protocol is labor-intensive. From a yet unpublished study comparing our LMD measurements taken in the week before the animals entered a RC, it has to be concluded that correlations with RC measurements were rather low as also indicated by (4). However, when methane emissions were expressed as g per day divided by kg ECM, correlations with RC measurements increased to 0.7 to 0.9 depending on diet. This new finding may therefore support the highly flexible LMD technology.

Methane emission data may also be recorded via indirect measurements, so called proxies. One example for this is the use of mid-infrared (MIR) spectra data routinely provided at time of analysis of samples in a milk recording laboratory. MIR data may be calibrated to predict methane emissions (5) and have the big advantage to be available for any cow under milk recording for several points in time per year. As this technology is the focus of another presentation at this workshop (6), it will not be discussed here.
For genetic studies, it has been recommended to use a phenotype defined as methane emissions in g/day (7) as this measurement is not dependent on air flow rates and also reflects the total of emissions not affected by variations within a day. Furthermore, a trait defined simply as the mass of methane is best suitable to be included in a selection index in contrast to other expressions as ratio traits which have body weight, dry matter intake, or milk production in the denominator. A very recent study (8) compares various definitions of a methane emission phenotype and recommends the use of g/day, corrected for ECM. This phenotype is uncorrelated with milk production on the phenotypic level although still genetically correlated with milk yield and thus suitable for a selection index.

Host genetic influences on the microbial population

In studies of the human microbiome, it has been established already quite some time ago that host genetic influences exist on the abundance of microbes in the intestine. Most of these studies have been conducted as twin studies (9, 10). In many studies, the 16S rRNA gene is used as the standard for classification and identification of microbes. In a study in cattle based on 72 steers measured for their methane emissions in RC and subjected to analysis of their microbiome in the rumen, it has been suggested to use the ratio of archaea : bacteria abundance as a selection criterion for methane emission and feed efficiency (11). Not all available studies using 16S rRNA technology and measuring methane emissions gave simple answers: In a study including 750 cows in commercial herds with sniffer methane emissions and quantification of microbial abundance (12), it was concluded that host genetic effects on the abundance of microbial populations could be proven with heritability estimates of around 20%, however, the association of microbial abundance with methane emissions was rather weak. In a recent study (13), it was suggested that rumen function and rumen productivity could be predicted from a core microbiome formed by microbial species which are independent of regional and dietary differences. The species belonging to this core show significant heritabilities and thus are influenced by host genetics.

Genetic parameters and genomic approaches

Classical genetic parameters for methane emissions in dairy cattle such as heritabilities have been reviewed by Lassen and Difford (14). Estimates of heritabilities mostly range between 0.05 and 0.26 with one exceptional estimate from a rather small study of 0.45. In the study of our group comprising 622 Holstein cows measured with the LMD (15), an estimate of 0.28 was obtained for the heritability of methane emissions defined as g/day. The aforementioned study (14) also reviews genetic correlations with traits other than methane emissions. In summary, genetic correlations with milk production, either defined as kg of milk, ECM, or FPCM, were substantial with magnitudes between 0.40 to 0.60 whereas genetic correlations with other traits as body weight, conformation traits, somatic cell score, and longevity mostly were between -0.30 and 0.30. Genetic correlations with dry matter intake varied between 0.08 and 0.60. Considering additive-genetic correlations, many correlations in many studies had high standard errors due to the small sample size which mostly was limited by the costs of obtaining methane emission data. Also, genetic correlations with many more traits are still missing, e.g. precise trait definitions of metabolic disorders and other health traits, fertility parameters, etc.
As early as 2009, efforts have been undertaken to identify genes within the cattle genome that have a substantial effect on methane emissions (16). Since 2008/2009, genomic selection based on genotyping for many single nucleotide polymorphisms (SNP) has been suggested and numerously applied in livestock populations. The prerequisite for genomic selection is the establishment of a large reference sample consisting of animals being phenotyped and genotyped. Once this reference sample is established and continuously updated with new animals, the benefit of genomic selection can be substantial, especially for traits difficult to record such as methane emission (7). Up to now, only few studies actually have been conducted running the full chain of necessary steps for genomic selection for methane emissions (17, 18). Accuracies for predictions of genomic breeding values have been low up to now but may increase with more data becoming available.

Conclusions

Host genetic effects on the microbiome of the rumen do exist and individual variation between cows for methane emissions is partly controlled genetically. Due to the large effort needed for precise measurements of methane emissions, a lot of uncertainty still exists. This imponderability pertains to the best trait definition, the most cost-effective yet precise measuring technology, genetic correlations with other traits of interest and the approach to be taken when methane emissions should be included in breeding objectives. Furthermore, it is still unclear whether an approach focusing on the rumen microbiome and the relative abundance of microbial species or a more classical approach directed towards host genetic influences and their associations with methane phenotypes is the best strategy for tackling emissions via breeding.

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Controlling methane emissions by feeding

Steuerung der Methanemission durch die Fütterung

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Controlling the causes and not only curing the symptoms – this is the principle behind applying dietary measures for mitigation of methane emissions in ruminants because nutrients, especially fibre, are the primary substrates for methane formation. Methanogenesis, where hydrogen – the product of ruminal and hindgut fermentation – is removed, is thus an indispensable element of the key to enable ruminants fitting perfectly into their niche which is to transform non-edible biomass into high quality foods. The globally too great number of domestic ruminants turned this natural process into an environmental problem. As no substantial global reduction of ruminant livestock number is feasible, feeding measures indeed get very important as mitigation measures in scenarios discussed to combat global warming. In cattle production systems, the mitigation potential of 30% is estimated which corresponds to 775 million tonnes CO_2 equivalents (methane = 23 CO_2 equivalents) (1). Feeding measures will remain important even when an efficient mechanism to genetically select low emitting ruminants may be in place in future (cf. contributions to this workshop by N. Gengler and H. Swalve), because these two approaches act additively. In the following, an overview of the state of knowledge about effective feeding measures for controlling methane emissions, i.e. a toolbox for this purpose, is given.

General aspects of changes in production efficiency

Flachowsky and Brade (2) stated (in German) in 2007 that „increasing animal performance and the concomitantly possible reduction of the number of ruminants should currently be the most effective methane mitigation option." In addition to theoretically needing less animals for producing the same amount of milk and meat, part of the well-digestible fibre in high-quality forage-based diets is replaced by starch from concentrate and, sometimes, less well digestible fibre from forages of lower quality. This seems very attractive, but the context is not as simple as it seems. On the level of large-scale economics, a reduction in animal number is not guaranteed in case of an increased performance. On the contrary, and different from what is generally assumed, an increase in demand for and use of foods from animals has to be predicted based on the Jevons paradox (3) in a situation where performance efficiency increases. This paradox states that in the history of human economies, an increase in efficiency in producing a product or achieving a good has hardly ever led to a reduction in resource use, because this increase is typically linked to an increase in the number of products or goods used in a human population. Additional problems are inherent to the production systems themselves. For instance, the same amount of milk produced from fewer dairy cows opens up a gap in beef supply which is typically filled by enhancing suckler beef production (4). Although being close to natural conditions, the latter system is characterised by a very low efficiency in producing edible animal protein. As a consequence, the greenhouse gas emissions resulting from the milk-beef system even tend to increase with increasing milk yield due to the co-product beef (4). In this context, according to Probst et al. (5) sperm sexing could improve the system efficiency because this technology enables a shift to beef cattle with higher growth potential than that of dairy calves.

Fibre vs. starch – or the most fundamental feeding system options and their difference in methane emission

As outlined above, with increasing dietary concentrate proportion less methane is expected to be emitted than with high-forage or forage-only diets. However, this attempt runs contrary to the general justification for keeping ruminants in the first place – that they can convert non-edible forages into valuable foods. In addition, it has to be noted that only very high proportions of concentrate really have a high efficiency against methane emissions, and this mostly because of a drop in ruminal pH to often non-physiological levels rather than because only of the exchange of fibre by starch. At moderate levels of concentrate, the methane reduction is modest (6). Recognising the low effect of moderate concentrate proportions, the IPCC decided to only distinguish between ruminant diets with less and more than 90% concentrate. In addition, the increase in methane emission from manure occurring concomitant to elevated concentrate proportions often remains unaccounted for. The reason for that is that the additional supply of the methanogens in the manure with extra undigested fibre from concentrate-based diets lowers their achievable net mitigation level further (7). On a wider system approach, other greenhouse gases have to be considered as well. Lorenz et al. (8) found that the carbon footprint of the production systems 'grazing', 'barn housing' and 'mixed systems' with average dietary concentrate proportions of 7%, 35% und 25%, respectively, did not differ substantially.

Increased feed conversion efficiency as a key to low methane emissions

On a closer look, the dilemma outlined above points towards the real key for low-methane/low-greenhouse gas system options. These have to build on an overall high feed conversion efficiency. This can be directly achieved by a higher diet quality from improving forage quality, but also from healthier animals and breeding for high feed conversion efficiency (a difficult but intensively discussed task). Another, less obvious attempt to improve system feed conversion efficiency is the strategy to aim at high longevity of the animals. A prerequisite for this option is the finding that older cows produce less methane per unit of feed consumed than cows in their third or fourth lactation (6). Even much more important is the amount of methane and total greenhouse gases saved by reducing the proportion of the time needed for rearing of replacement animals, i.e. the time until eventually milk is produced (9). An approach to better describe system efficiency could, therefore, be milk yield per day of life rather than the actual milk yield per day or per lactation. Again, this works only under the assumption that an increase in the cows' longevity will be accompanied by keeping animal numbers stable.

Methane mitigation by the strategic use of non-starch-non-fibre nutrients

Fats, oils and fatty feeds.These are the classics among the methane mitigating approaches. There is a close dose-response relationship, but some fatty acid exhibit specific anti-methanogenic properties. This was first demonstrated for coconut oil, rich in lauric and myristic acid (both medium-chain saturated fatty acids) in 1999 (10), where methane emissions of sheep were lowered by up to 73% compared to that found with a control diet. In subsequent studies it turned out that the efficiency of this lipid (and likely other lipids) was lower in forage-based diets (coating of fibre) and in diets supplemented with extra calcium (soap formation) (11). Apart from these fatty acids, which are considered problematic from a human nutrition point of view, unsaturated fatty acids, especially poly-unsaturated fatty acids have a specific efficiency against the methanogens. Therefore, in the last decades, there was an intensive search for lipid sources which can be sustainably produced and best have other favourable effects, too. These sources were found in the oilseeds which need to be provided in crushed or extruded form to exhibit a mitigating effect. Especially the full-fat linseed got specific attention due to its richness in α-linolenic acid, one of the valuable n-3 fatty acids, the feeding of which enhances its content, and those of conjugated linoleic acids of cow's milk (12). Other interesting oilseeds are rapeseed and, less so, sunflower seed and full-fat soybeans (both rich in n-6 fatty acids). Much less well known are oilseeds from safflower *(Carthamus tinctorius)*, poppy *(Papaver somniferum)*, hemp *(Cannabis sativa)* and camelina *(Camelina sativa)*. Especially the first two seeds also turned out to have methane mitigating properties *in vitro*, and both, hemp (admitted for feeding to livestock except to milk producing animals in Switzerland) and camelina are rich sources of n-3 fatty acids, too (13).

Sugars. Another group of nutrients typically low in ruminant diets, sugars, might also be effective. 'Sugars' are defined here as the entire group of the water-soluble carbohydrates. In case sugars replace fibre in the diet, they may reduce methane formation as there is less of the main precursor, fibre. Examples for ruminant feeds rich in sugars are beet pulp, high-'sugar' grass cultivars and whey from cheese production. In Weihenstephan, already in the 1990ies balance studies were performed with dairy cows fed large amounts of beet pulp instead of maize grains. This exchange (14), similar to the use of up to 5 kg/day of pure sugar (15), did not mitigate methane production of the cows. Similarly, the use of high-'sugar' grass hay with 19% instead of 10% soluble carbohydrates in a control hay did not modify methane emission in dairy cows (16). Common to all three mentioned studies was that no fibre was replaced by the sugars in the diet. Different from that, Dufey et al. (17) reported in the 2019 GfE conference that liquid whey, rich in lactose, reduced methane emissions by almost 40% when offered to heifers. In that study, the heifers consumed 2.3 kg of whey and 6.4 kg of grass per day (on a dry matter basis). Further studies have to show whether this substantial mitigation can be repeated or whether there is a partial compensation by an enhanced hindgut fermentation (measurements were made with the SF_6 method which does not register hindgut methane). In all these studies with high sugar supplementation quoted, no cases of rumen acidosis were reported even though all diets had been fed to ruminants with fully functional rumen.

In conclusion, fats and sugars seem promising both as nutrients and methane suppressors. However, it always has to be kept in mind that these nutrients are not prominently represented in natural diets of ruminants and, therefore, caution is indicated when aiming at a large methane mitigating effect.

Methane mitigation by supplementation with natural feed components

Feed supplements from natural resources containing effective plant secondary compounds (PSC) are currently intensively researched in nutritional methane mitigation attempts. As the number of potentially effective plants and PSC is immense, various *in vitro* screenings have been undertaken. One well-known large screening is the concluded "Rumen Up" project (www.abdn.ac.uk/research/rumen-up). Promising plant additives have to be tested *in vivo* in the animal with respect to the following key objectives: 1) efficiency in methane mitigation, 2) sufficient palatability and 3) no or only few anti-nutritional effects like a reduced digestibility. The latter two determine their usefulness in feeding practice, where also cost-effectiveness plays a major role. However, once the use of distinct efficient PSC sources becomes common practice, costs may substantially decrease due to economy of scale in production.

Tannins. The best researched class of PSC is that of the tannins, which are large and complex molecules. A meta-analysis demonstrated a clear dose-response relationship, but also that at low (below 2%), and therefore low-cost, dosages no reliable effects can be obtained *in vivo* (18). Similar to the fat sources, there are differences between tannin sources in mitigation efficiency, palatability and effects on digestibility. Therefore, the threshold in dosage where adverse effects outweigh the favourable effects depends on the tannin source and cannot be generalised. One source proven repeatedly (but not always) to be efficient is the extract of the bark of an acacia species *(Acacia mearnsii)*. This extract is produced in large amounts for industrial tanning of leather. The first proof of principle was made in lambs in 2005 (19). A more recent breakthrough was the demonstration of its long-term efficiency (>6 months) in fattening bulls (20), a property which not all of the discussed methane suppressing supplements may have. Recently, it could even be demonstrated that *A. mearnsii* has an immediate anti-methanogenic effect (21). Thus, having long-term and quick effects, and the mostly maintained animal performance make this product particularly attractive. Still, this feed supplement has to be imported, and sources from on-farm cultivation would be preferable for a sustainable application. Tannin-rich forages which can be grown on arable land under temperate climatic conditions include for instance the legumes sainfoin *(Onobrychis viciifolia)* and birdsfoot trefoil *(Lotus corniculatus)*. Although being efficient in mitigation of ammonia emission from the manure, similar to most other tannin-containing supplements, no significant effect on methane emission was found *in vitro* (22). There are more potential tannin sources to be found in feeds from herbs, shrubs and trees from European origin. A recent screening with the Hohenheim gas test identified six particularly promising plants (23), whereof four turned out to be highly palatable in dairy cows, namely leaves from hazel *(Corylus avellana)* and green grape vine *(Vitis vinifera)* and the herbs rosebay willow *(Epilobium angustifolium)* and wood avens *(Geum urbanum)* (24). For hazel leaves a reduction of the methane emission in sheep of up to 35% was found; the latter, however, only with a dietary hazel proportion of 50% (25). Lower dosages of hazel leaves were also effective, and in dairy cattle a close dose-response relationship was described recently (26).

Saponins. Another group of compounds of interest are saponins. Extracts or plants rich in saponins, prevalent in a number of plants especially in the tropics, have been repeatedly shown to be effective (e.g. 27). However, saponins may be toxic in high amounts, and therefore the balance between a significant methane suppression and lack of adverse side-effects is delicate which makes their practical application difficult.

Essential oils. A number of researchers focus on another group of PSC, essential oils or plants rich in such oils. Essential oils from thyme, oregano and cinnamon or their principal components were supplements which showed promising methane mitigating results; but, so far, products based on essential oils with proven high efficiency against methane are scarce and the knowledge about their active compounds and effective dosages is limited (28).

Sulphur-containing compounds. Sulphur containing compounds also have been specifically investigated. Especially garlic contains such compounds (apart from essential oils), and they are responsible for the specific garlic smell. Using garlic oil *in vitro* in Rusitec resulted in a methane suppression of 91% of a control (29). The effect of garlic oil was, however, much smaller in sheep (30) and 3% garlic bulbs in the diet did not significantly mitigate methane in fattening bulls (20). Another restriction is that garlic-based feeds are not permitted in dairy cow nutrition, at least, in Switzerland.

Bromoform. Recently an unconventional source of feed for ruminants received a very high attention: A certain species of red macroalgae, Asparagopsis taxiformis (a seaweed), was found to have drastic effects against methane, reduction by 99% of control *in vitro* (31) and by 80 % when fed at 3% of the diet to sheep (32). The effect seems to rely on a specific halogenated compound, bromoform, which is prevalent in these algae and reacts with a vitamin B_{12} cofactor, essential for methanogens. However, safety of its use in food producing animals is still unclear and needs further investigation.

A final remark to the anti-methanogenic measures based on PSC: these sources are natural products with often underestimated natural variation. This means a limited reliability of having a certain level of effect, sometimes even of having the effect at all. Still, the knowledge about an average efficiency helps to decide about their use in farm practice and for any financial or greenhouse gas compensatory schemes.

Methane mitigation by supplementation with synthetic feed components

Methane inhibitors are pure chemicals, often analogous to methane, and as such cause a negative feed-back to methane synthesis. They are known for long and may inhibit methane formation to close to zero (e.g. by 85% with bromochloromethane; 33), but they are not applicable in farm practice. Certain feed antibiotics (not allowed for use in Europe), especially monensin, are praised to have anti-methanogenic properties, but the level of effect is far from being inhibitors (34) and possibly transient. In order to avoid the stigma as chemical feed additives, the search now concentrates on compounds found in nature, but which also can be chemically synthesised. Among them, fumaric acid turned out to be rather effective (reduction by 76% of control when fumaric acid was provided in an encapsulated form) (35), but this product is very expensive. Also allyl isothiocyanate (29) and diallyl disulphide (30, 36), two sulphur-containing garlic compounds, have exhibited a high efficiency *in vitro* (reduction by 68% of control) when applied as pure substances; however, the efficiency of these compounds to mitigate methane emissions in sheep was limited. In the context of compounds occurring in nature, 3-nitrooxy propanol (3-NOP) triggered a large number of experiments after its detection. It is currently considered highly promising as its efficiency against methane is among the highest ones found with feed additives (by 10 to 40% of the methane amount compared to control; e.g., 34 and 37) This compound influences the methyl-coenzyme M reductase, a key enzyme of the methane forming pathway of the methanogens (37). Its admission as feed supplement has been applied for by the producer in the EU and in USA.

Same diet, same CH_4 emission?

A final chapter shall be devoted to a recently discovered phenomenon of a possible genotype × nutrition interaction, or the lack of such an interaction. On a broader scale, when fed on the same diet, there is an astonishingly similar level of methane yield per unit of feed ingested both among various ruminant species and among a large number of non-ruminant herbivore species (the latter produce methane at a level of about one quarter of that emitted by ruminants) (38). However, on closer inspection, there seems to be individual, possibly genetic, differences between animals which are expressed as a lower methane formation per unit of digested fibre along with higher fibre digestibility. This was first described in a dataset from one experiment focussing on the methane emission of cows at different ages (39). This dataset showed that, although the mean retention time was increased with an increasing fibre digestibility, less methane was produced per unit of digested fibre despite the same diet was fed. Further analysis of a great number of experiments confirmed this negative relationship between fibre digestibility and CH_4 yield per unit of digested fibre (40). Clarifying the causes of this phenomenon, and if and how it could be implemented as a methane mitigation tool, warrants further studies.

Conclusion

The list of potential measures to be added to the 'toolbox' for nutritional methane mitigation is quite extensive. Efficiency, price and the side-effects especially on performance vary largely. It is likely that there is not the *one* measure which is suitable for all farms around the globe. It is preferable to look for tailor-made solutions for individual countries, regions or even farms. The individual mitigating measures also have to be analysed for their effects on other types of emissions like those of nitrogen as nitrous oxide, ammonia or nitrate. Some are efficient against more than one type of emissions (e.g. tannins), others not (e.g. lipids). In addition, any carbon footprint associated with production and distribution of supplements designed to mitigate methane emissions must be considered in scenarios about their usefulness. To date, it is not realistic to expect a relevant increase in production efficiency for milk or meat in parallel with methane mitigation – on the contrary, it is typically considered a success if a mitigation measure does not reduce production efficiency. Due to the misbalance of the costs for implementing an efficient measure and the common good of a global environment, it is difficult to imagine that nutritional measures will achieve a widespread application without incentive-driven policies. Such incentives could consist in compensation schemes, fines ('methane tax'), and higher-priced labelled foods in the market. The urgency of the debate about how to best counteract climate warming may well trigger such policies. In this context, it is helpful that measures in the area of

animal production systems are characterized by the possibility of implementation in the short-term and by a quick effect on global warming due to the far shorter half-life of methane in the atmosphere compared to carbon dioxide.

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