Proceedings of the Society of Nutrition Physiology • Band 28, 2019

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Berichte der Gesellschaft für Ernährungsphysiologie

Band 28, 2019

DLG Verlag

Proceedings of the *Society of Nutrition Physiology*

Berichte der Gesellschaft für Ernährungsphysiologie

Editor: Society of Nutrition Physiology
Herausgeber: Gesellschaft für Ernährungsphysiologie

Volume 28, 2019
Band 28, 2019

73rd Conference 13th–15th March 2019 in Göttingen
73. Tagung vom 13.–15.03.2019 in Göttingen

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

Deutsche Bibliothek - CIP Einheitsaufnahme

Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.dnb.de abrufbar.

ISBN 978-3-7690-4112-5

Redaktion:	D. Kampf, S. Ausmeier Gesellschaft für Ernährungsphysiologie Eschborner Landstraße 122, 60489 Frankfurt am Main
Herstellung:	D. Schirach, DLG-Verlag, Frankfurt am Main
Layout:	P. Kapischka, N. Suchowsky, event lab. GmbH, Leipzig
Druck:	L&W Druck, Bad Endbach

Conference abstracts were peer-reviewed.

Members of the reviewer panel were:

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

W. Windisch
Chairman

Contents

Review-Lecture

Metabolic imprinting by pre- and early postnatal nutrition in farm animals

Metabolische Prägung durch prä- und frühe postnatale Ernährung beim landwirtschaftlichen Nutztier

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Modern animal production is mainly based on economic principles; animal welfare and ecological issues are less considered. Short time spans between birth and weaning, rapid rearing of young farm animals and an early use for reproduction are common. Such an acceleration of physiological developmental processes is against the evolutionary conserved principle of growth and development of mammalian species.

About 300 million years ago, an exciting biological development began to form mammalians, which was connected to designing a placenta and to establishing milk secreting glandulae with teats in the skin. Furthermore, prerequisites for a functional mammalian animal were endothermy (homeothermy) and isolation of the body by fat and fur. Besides others, those characteristics enabled more continuous activity and life in mammalians compared to poikilothermal animals. About 65 million years ago, this form of modern mammalian life was dispersed over the whole world with only few exceptions.

Regarding reproduction, the fertilised egg was now developing in the mother´s womb and under the influence of the maternal nutrition and metabolism it reached maturity for birth. The early postnatal period was similarly mother-bound, the milk produced by the maternal mammary gland was developed to match all needs of the offspring regarding energy and substrates. The close relationship between mother and offspring was protecting the young against predators and hunger. A calf doubled its life weight from birth on within 48 days nourished only with milk; a piglet needed 13 days to double its birth weight. This demonstrates the exceptional high biological value of mother´s milk. Furthermore, plenty of bioactive components, microbiota and cells in the milk support a healthy postnatal development of human and animal offspring; human breast milk contributed directly to infant´s innate immunity and maturation of the intestinal barrier function (1).

Influence of prenatal nutrition on metabolic health of offspring

Even before birth, the nutrition of the mother is a well-known factor to influence metabolism of the unborn. Prenatal stress during gestation was identified as major problem in modern farm animals (2). Intrauterine growth restriction (IUGR) was a commonly observed acute consequence of malnutrition in the mother. However, also caloric overnutrition was affecting the unborn directly. However, these disturbances in the maternal nutrition could also have a long-lasting influence on the metabolism of the offspring with life-time consequences, a process which was called nutritional programming (3) - thrifty phenotype hypothesis), fetal programming (4), developmental programming (5) or metabolic imprinting (6). The dairy cow, which is increasingly used for milk production at very early age (first calving age of 20 to 24 month), most likely created an endogenous malnutrition for the offspring due to sharing resources between the still growing mother and the unborn calf. Furthermore, high producing dairy cows were pregnant most of their productive time; thus the unborn calf had to share resources with the mammary gland of the mother. Both may imprint the metabolism of the offspring in later life.

Many studies about effects of maternal nutrition on intrauterine development of the embryo and fetus were done in sheep resulting in identification of nutritionally sensitive time windows for metabolic imprinting (4). Key periods of development, which are nutritionally sensitive, are within the embryonal, the placental and the fetal life time of the offspring. During embryonic development the brain and the cardiovascular system were especially sensitive to nutritionally challenges, while shortly after implantation, during early placental period, the kidney was the mostly affected organ. During the fetal period from about day 85 up to birth, metabolism of adipose tissues and of muscle was highly responding to nutritional challenges (4).

Early dietary restrictions during embryonic period in sheep resulted in smaller brains and behavioural changes with a higher emotional reactivity and impaired cognitive flexibility (7). Maternal nutritional restriction during the last third of pregnancy inhibited fetal growth strongly. Birth weight of IUGR lambs was reduced; they expressed catch up growth and adiposity at 1 year of age. Concomitantly, glucose/insulin and lipid metabolism disturbances occurred in the young adult sheep (8). Heifer calves born from primiparous and multiparous mothers differed significantly in birth weight with lower weights in calves of heifer mothers (9). These calves were prone to develop catch up growth as well. IUGR rats with undernutrition during their whole intrauterine life expressed changes in the endocrine system. The mismatch between intrauterine and postnatal nutritional level was resulting in a storage-type metabolic condition with increased adiposity

and insulin resistance (10, 11). Furthermore, at birth, they had lower leptin concentrations which increased significantly during growth resulting in hyperleptinemia, central and peripheral leptin resistance and lipid metabolism disturbances in adulthood (12, 13). Leptin is known to be one of the most important hormones for regulating energy metabolism and food intake of a young mammalian (14). Lower birth weight and diminished leptin concentrations around day 10 of life were also observed in female Holstein calves born by heifer mothers compared to calves born from multiparous mothers indicating that immaturity of the mother could also result in IUGR (Schwarzkopf et al. 2019, unpublished). They consequences of being a heifer calf in later life is under scientific evaluation right now. Identification of novel metabolic markers for metabolic imprinting is under progress by targeted metabolomic approaches using the IDQ p180 panel (Biocrates, Innsbruck). This panel enabled HPLC/mass-spectrometry-based quantification of hexoses, acylcarnitines, amino acids, biogenic amines, glycerophospholipids and sphingolipids, metabolites which reflect status of glucose and lipid metabolism, of insulin resistance, of mitochondria functionality and of systemic low grade (metabolic) inflammation. However, using this targeted metabolomics approach, acylcarnitine profiles of calves at day 10 of age grouped independently of parity of mothers. Further other factors must be relevant to determine metabolic profiles of calves early in life. Acylcarnitines resulted from mitochondria fatty acid metabolism; thus grouping of certain calves according to their acylcarnitine profile may indicate differences in mitochondrial functionality.

During very early evolution, bacteria with a capacity for oxidative phosphorylation invaded into eukaryont cells and a close symbiotic life started since then. Cells obtained the advantage to efficiently generate ATP by oxidative metabolism. Due to that history, mitochondria kept their own genetic material, mitochondrial (mt) DNA, a small DNA double ring of 16.3 kb of size. It codes for 37 genes, 13 of them are translated into enzymes of the respiratory chain. From the nuclear genome, all other mitochondrial proteins were provided. Recently, it was discovered, that individual metabolic profiles in dairy cows were associated with certain mitochondrial DNA haplotypes (15). While mtDNA haplotype 2 was associated with high hepatic fat accumulation and low acylcarnitine levels in plasma, haplotype 4 with low liver fat expressed higher plasma acylcarnitine concentrations. Hypothetically, calves at day 10 of age may carry different mtDNA haplotypes and thereby, variations in mitochondrial functionality occurred. During reproduction, since the source of mitochondrial DNA is located in the oocyte, the mother is responsible for transfering this genetic information into the offspring. And, to make it more complex, nuclear and mitochondrial genome obviously need to match (16). Nuclear and mitochondrial genetic background of the calf in connection with intrauterine or postnatal dietary challenges may imprint metabolic health in later life. Thus, maternal influence on metabolic health should be considered in breeding concepts of the future.

Influence of early postnatal nutrition on metabolic health of offspring

As discussed before, intrauterine nutrition of heifer calves and calves of high performing mother cows face intrauterine malnutrition due the competition between the needs of the growing or performing mother and of the developing offspring. Furthermore, early postnatal life is also a critical time window in which metabolic imprinting can occur. Calves ingested up to 15 L milk replacer in 9 -11 meals per day if supplied ad libitum (9, 17). Any lower amount of milk or milk replacer can be defined as restrictive feeding which pushes the calf to ingest solid feed although it is physiologically immature. Gastrointestinal maturity is reached at about 14-16 month of life (18). The observation of “compensatory growth” after weaning in former restrictively fed calves was used to claim that the young animals were adapting properly to the feeding conditions. A pre-ruminant status was defined to exist only within the first 3 weeks because during that time calves did not ingest solid feed even when milk supply was restricted to 5 L per day (9). Most likely, these calves are starving to death, thus they decide to eat solid feed to escape the life-threatening situation – although their ontogenesis is not finished. To calculate feed efficiency in calves - feed per growth - is against the physiology of early development. Hastening the development of a young animal results in resource allocation mismatch and disproportional growth (19).

Restriction of milk intake in a very early period of postnatal life imprinted metabolic conditions of offspring sustainably as assessed by a targeted metabolomics approach. Calves fed restricted between day 4 and day 27 of life expressed significant changes in acylcarnitine concentrations throughout their later life until first parturition as heifers. Ad libitum fed calves expressed higher concentrations of acylcarnitines (6). These metabolites were identified to be associated with a longer productive life span and indicated a well-developed mitochondrial functionality in adult dairy cows (20).

Conclusion: Pre- and early postnatal nutritional requirements of young mammalians must be reconsidered strongly respecting the physiology of a developing young animal. Only fully developed and mature farm animals will be able to stay healthy for a long productive life and to express high metabolic performance.

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Abstracts

1.

Excretion of inositol phosphates in dairy cows fed soybean meal or rapeseed meal-based diets

Ausscheidung von Inositolphosphaten bei Milchkühen bei Fütterung von Rationen mit Soja- oder Raps-extraktionsschrot als wesentliche Proteinkomponenten

Haese E., Gänger K., *Rodehutscord M. – Stuttgart-Hohenheim

The ruminal degradability of inositol phosphates (InsPs), especially that of phytate ($InsP_6$), differs strongly between soybean meal (SBM) and rapeseed meal (RSM). At a ruminal outflow rate of 0.08 h^{-1}, effective ruminal $InsP_6$ degradation was 74% for SBM and 50% for RSM (1). Although post-ruminal $InsP_6$ hydrolysis can occur and contribute to total tract disappearance of $InsP_6$ (2), the amount of $InsP_6$ excreted with faeces is mainly determined by the extent of ruminal $InsP_6$ hydrolysis. The objective of this study was to examine if diets based on SBM or RSM lead to differences in total tract disappearance of InsPs in lactating dairy cows and if $InsP_6$ disappearance is affected by feed intake (FI).

Methods: Fourty five lactating Holstein cows were divided into two groups by building 22 pairs regarding lactation number and stage. The remaining animal was randomly assigned to one group. Two diets (diet SBM and diet RSM) consisting of 22% maize silage, 16% grass silage, 18% hay, 3% straw, 12% maize, 5% barley, 2% mineral vitamin mix and 22% RSM or SBM (on DM basis) were prepared daily and fed as TMR. Urea was added to diet RSM to achieve equal N concentrations in both diets. TiO_2 was used as indigestible marker to quantify faecal excretion. After an adaptation period of 14 d, faecal grab samples were taken twice daily after milking (5 am; 4 pm) from each cow for 7 d and frozen immediately (sampling period one; SP1). After 4 weeks of continued feeding of the experimental diets, again samples were taken for 7 days (SP2). For each sampling period, samples were pooled per cow for analysis of InsPs and Ti. InsPs were analysed with high-performance ion chromatography after extraction with an EDTA-sodium fluoride solution. Throughout the experiment, FI was recorded daily for each cow individually. TMR samples were taken every day, frozen immediately and pooled for each TMR at the end of the experiment for analysis. A bivariate mixed model approach was used to evaluate the effects of diet, SP, and $InsP_6$ intake on total tract disappearance of $InsP_6$. Furthermore, linear and quadratic effects of feed intake on $InsP_6$ disappearance were evaluated.

Results: The $InsP_6$ concentrations of the diets were 3.76 g/kg DM (diet SBM) and 6.86 g/kg DM (diet RSM). Feed intake was not influenced by diet but was significantly higher in SP2 (26 vs. 25 kg DM/d). No effects on $InsP_6$ disappearance were observed for FI, neither within diet nor within SP. Higher $InsP_6$ intake, as effect of diet or SP, led to significantly higher $InsP_6$ excretion and reduced total tract $InsP_6$ disappearance (89% SP2 vs. 91% SP1; 87% diet RSM vs. 93% diet SBM). Total tract disappearance of $InsP_6$ for diet RSM ranged from 81-93% between cows, while lower variation was observed between animals fed diet SBM (90-96%). For diet SBM, no lower InsPs ($InsP_{3-5}$) were detected in the faecal samples while small amounts of $InsP_5$ (< 0,4 g/kg DM) occurred in some samples of cows fed diet RSM.

Conclusions: The higher FI in SP2 might have led to higher passage rates and reduced time for ruminal $InsP_6$ hydrolysis thus decreasing total tract disappearance of $InsP_6$. Furthermore, a limitation of microbial phytase activity in the rumen might have occurred with increased $InsP_6$ intake. This also might apply for the observed lower total tract disappearance of $InsP_6$ for diet RSM. However, the high variation of $InsP_6$ disappearance between animals for diet RSM could also indicate that the individual microbial composition in the rumen is more relevant when $InsP_6$ with low effective ruminal degradability is fed. No accumulation of lower InsPs occurred in the faeces confirming earlier conclusions that in ruminants, the cleavage of the first phosphate group is the decisive step in ruminal InsPs degradation (3).

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2.

The addition of phosphorus and calcium alters the caeca microbial diversity and functionality of broiler chickens

Die Ergänzung von Phosphor und Calcium beeinflusst die mikrobielle Diversität und Funktionalität im Caecum von Broilern

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Mineral supplementation of phosphorus (P) and calcium (Ca) is a crucial concern in broiler chicken nutrition. In the interaction between both minerals, the chemistry of the intestinal content is modified [1]. This modification will have an impact on the microbial community distribution and its functional capabilities [2]. Thus, this study aimed to investigate through taxonomical and functional approach, the disturbance of the microbiome under the influence of mineral supplementation.

Methods: A low-P (4.1 g/kg) low-Ca (6.2 g/kg) maize-soybean basal diet was used as a control and two supplemented diets included the addition of P (2 g/kg) and Ca (3 g/kg). Broilers were fed with a commercial starter diet until day 14 and then pens were randomly assigned to the dietary treatments. On day 26, birds were euthanized by carbon dioxide following anesthesia in a gas mixture, and both digesta and mucosal samples were taken from the caeca of three birds per treatment. Total nucleic acids from individual bird samples were extracted using a commercial kit and then subjected to 16S Illumina amplicon sequencing and whole genome shotgun sequencing (metagenomics). Samples have been analyzed in triplicates, due to the high costs of metagenome sequencing. Phylogenetic analysis of the 16S rRNA gene sequences was assessed using RDP pipeline [3], and metagenome analysis was done through an in-house pipeline. A multivariate statistical analysis which includes PERMANOVA routine was carried out to both phylogeny and functional datasets [3].

Results: A significant difference in the microbial community structure of digesta and mucosa was observed in all diets. Lachnospiraceae was the most abundant family, in the treatment supplemented with P in both types of sample (33% in digesta and 37% in mucosa). In the Ca supplemented diet, the presence of Peptococcaceae (11% and 8%, respectively) increased compared to the other treatments. The digesta from birds fed the basal diet revealed a high abundance of the family Erysipelotrichaceae (14%) while in the mucosa the family Anaeroplasmataceae was more detected (10%). Metagenome genes were classified into four categories to describe the functional potential of the microbiome influenced by the dietary interventions. At the broad category, for both luminal content and mucosa, the metabolite information category registered higher percentage of abundant genes (approx. 50%) in the supplemented diets while in the basal diet it was only 40%. Moreover, the basal diet comprised more information for the category genetic and information processing (44%) in comparison with supplemented diets which decreased the values to 10%. A further step in the functional classification, registered in the supplemented diets an increase of amino acid metabolism (17%) and carbohydrate metabolism (15.7%), compared to the control (12.4% and 13.7% respectively). As the fourth category was seen mainly represented in the digesta samples, in there, metabolic pathways were assigned in higher abundance to enzymes related to butyrate production, nitrogen metabolism and phosphate metabolism for the supplemented diets.

Conclusion: Changes in mineral supplementation resulted in modifications of microbial composition in the caeca, which impacted the assigned functionality with more genes encoding for the metabolism in the mineral added diets. This fact indicates that a higher concentration of P and Ca-activated metabolic fluxes, while the absence improved the gene abundance of genetic information related activities in the basal treatment.

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3.

Effects of lactic acid treatment of cereals and dietary phytase on calcium and phosphorus balance and serum parameters in growing pigs

Der Einfluss von Milchsäure-behandelten Getreide und Phytase auf die Calcium- und Phosphor-Bilanz sowie Serumparameter beim wachsenden Schwein

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The positive effect of dietary phytase on phosphorus (P) digestibility in pigs is well established (1). Soaking of cereal grains in lactic acid (LA) recently showed to reduce the phytate-P content in wheat and corn. However, it has not been evaluated *in vivo* whether this P release is similar to the action of phytase or may potentiate the effect of phytase and therefore have the same positive effect. In the present study, we investigated the effect of LA-treatment of cereals and dietary phytase on performance, soluble P fraction in the stomach, calcium (Ca) and P balance, and serum parameters in growing pigs.

Methods: Thirty-two castrated male crossbred fattening pigs (13.1 ± 2.3kg) were randomly assigned to one of four diets in a completely randomized design in four 18-day replicate batches, with a total of eight observations per diet. The diets were: Con) diet with untreated wheat and corn and no phytase; Con-Phy) diet with untreated wheat and corn and phytase supplementation; LA) diet with LA-treated wheat and corn and no phytase; LA-Phy) diet with LA-treated wheat and corn and phytase supplementation. Feces and urine were collected from day 15 to 17 and blood samples were collected on day 18. Titanium dioxide was used as digestibility marker. The pigs were individually housed and fed semi-*ad libitum*. Data were subjected to ANOVA using the Proc Mixed procedure of SAS with the fixed effect of treatment and the random effect of replicate batch and differences at $p < 0.05$ were considered significant.

Results: Feed intake and average body weight gain were similar in all diet groups. The LA-treatment of cereals tended to improve the feed conversion ratio by 6.7% ($p < 0.1$). The percentage of soluble P in the stomach was increased by 6.0% in the Phy diet groups ($p < 0.001$). The phytase × LA interaction ($p = 0.001$) for soluble P showed that this effect was further enhanced by 11.3% in the LA-Phy diet group compared to Con-Phy diet group. Overall, phytase increased ($p < 0.001$) the retention of Ca and P by 40.3 and 26.9%, respectively. Both phytase and LA-treatment of cereals decreased total P excretion ($p < 0.001$), which is mainly due to the lower fecal P excretion with both dietary treatments ($p < 0.05$). In contrast, phytase increased urinary P excretion ($p < 0.05$). Moreover, both LA-treatment of cereals and phytase decreased fecal Ca excretion ($p < 0.05$). However, there was an opposite effect of phytase and LA on urinary Ca excretion which was increased with LA but decreased with phytase ($p < 0.01$). As a result, only phytase reduced the total Ca excretion ($p < 0.001$). Serum Ca was increased by 9.1% in the LA diet groups ($p < 0.05$), whereas serum P was increased by 21.1% in the Phy diet groups. Phytase lowered serum fibroblast growth factor 23 (FGF23) by 9.8% ($p < 0.05$), whereby the phytase × LA interaction ($p < 0.05$) indicated that the decrease of FGF23 was greater in the LA-Phy diet group than in the Con-Phy diet group. Serum vitamin D was increased by 19.5% in the Phy diet groups ($p = 0.001$) but not in the LA diet groups. Serum osteocalcin, in turn, was not affected by LA-treatment of cereals or phytase.

Conclusion: Although the LA-treatment of cereals enhanced the gastric soluble P fraction, only phytase was efficient to improve the P retention. The increased retention and higher serum levels of P with phytase may have caused the higher serum vitamin D levels to stimulate Ca re-absorption. The interactive effect of phytase × LA on serum FGF23 may support different regulatory mechanisms to maintain Ca and P homeostasis with the two dietary treatments, which may have been related to the intestinal uptake and systemic usability of Ca and P.

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4.

Growth performance and copper status of weaned piglets fed two copper sources

Wachstumsleistung und Kupferversorgung von abgesetzten Ferkeln beim Einsatz von zwei verschiedenen Kupferquellen

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In European countries, the maximum concentration of copper (Cu) in pig diets is 25 mg/kg of complete feed, except for weaned piglets: up to now, Cu can be supplemented at supra-nutritional level (160 mg/kg) for these animals. Indeed, a high dosage of Cu improves growth performance of piglets after weaning. New legislation reduces the maximum concentration of Cu to 150 mg/kg up to 4 weeks after weaning, thereafter 100 mg/kg up to 8 weeks after weaning. Copper sulphate ($CuSO_4$) is commonly used, but new sources are under investigation, as copper(I) oxide (Cu_2O) which has been recently approved in the European Union.

Methods: Experimental diets were supplemented with 15, 80 or 160 mg Cu/kg of diet, from $CuSO_4$ or Cu_2O (CoRouge®). For this experiment, 600 piglets, weaned at 26 days, were divided among 60 pens (10 piglets per pen, 10 pens per treatment). Growth performance was measured at day 14 (end of the pre-starter phase) and at day 35 (end of the study). At the end of the trial, 8 piglets per treatment were sacrificed. Copper concentration in plasma, in the liver and in the bile were measured. Gut tissues were sampled and expression of genes related to metal transport were determined by RT-qPCR.

Results: A dose-response effect was observed for growth performance: ADG increased as Cu dose increased ($P < 0.01$), while feed conversion ratio decreased ($P < 0.01$). Cu_2O tended to increase ADG ($P < 0.09$) more than $CuSO_4$, especially at the lowest dose. The mode of action of Cu at high dosage is not fully elucidated, but its antibacterial properties could explain its positive effect on diarrhea of post-weaning piglets. In addition, the solubilization of Cu_2O releases cuprous ions Cu^+ (instead of cupric ions Cu^{2+} ions for $CuSO_4$); *in vitro*, antibacterial properties of Cu^+ are higher than antibacterial properties of Cu^{2+}.

Cu concentration in plasma remained constant, regardless of the source and dosage in the diet. Liver Cu-content and intestinal MT1A increased more with incremental dietary $CuSO_4$ then with Cu_2O (interaction $P < 0.01$). These results suggest that the absorption kinetics of copper sources could be different, probably related to their ionic forms (cuprous or cupric). The Cu content in the bile increased significantly in the groups fed 160 mg/kg of Cu, for either Cu source.

Increasing the dosage of Cu increased the expression of intestinal DMT1, numerically (Cu_2O) or significantly ($CuSO_4$).

Conclusion: In conclusion, Cu_2O can enhance growth performance, with a different effect on Cu accumulation in organs and in expression of certain genes compared to $CuSO_4$. Further analyses are in progress.

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5.

The effects of high dosages of zinc on porcine T-cells

Effekte von hoch-dosiertem Zink auf porzine T-Zellen

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Zinc serves as a powerful feed additive to reduce post weaning diarrhoea in pigs. However, mechanisms responsible for zinc-associated effects on the adaptive immune response following a very high dosage of zinc feeding remain elusive. Our aims are to investigate (i) if zinc transporters are expressed on immune cells, and (ii) if the adaptive immune cells of gut associated lymphatic tissues (GALT) respond to and are affected by feeding of zinc in a dosage-dependent manner, and (iii) how GALT is influenced by the exposure duration to different zinc dosages.

Methods: To reach our aims, we examined the relative cell counts of T-cells and the relative expressions of zinc transporters and immune relevant genes in gut associated lymphatic tissues of 72 weaned piglets fed diets with 57 (low, LZn), 164 (medium, MZn) or 2,425 (high, HZn) mg Zn/kg feed for one, two, or four weeks. Therefore, a total of 72 purebred landrace piglets were weaned at 26 ± 1 days of age with a mean body weight of 7.2 ± 1.2 kg and randomly allocated into three dietary treatment groups balanced for gender, litter and body weight. Animals were housed in pens (n = 2 per pen) with straw bedding and *ad libitum* access to feed and water. Each dietary group (n = 24 per feeding group) was fed a common basal maize-wheat-barley-soybean diet with analytical grade ZnO (Sigma Adrich, Taufkirchen, Germany) added to obtain the different Zn levels.

Results: Expression of the long *ZIP4* transcripts ZIP4-201 and ZIP4-001 and of the zinc transporters *ZnT1*, *ZnT2*, and *ZnT5* were measured in mesenteric lymph nodes and Peyer´s Patches. Our research provides evidence that *ZIP4*, *ZnT1*, *ZnT2*, and *ZnT5* are expressed not only in epithelium but also in mesenteric lymph nodes and Peyer´s Patches of the jejunum. However, no significant expression differences in these lymphoid tissues among the feeding groups for any examined zinc transporter gene was detected. A slight increase of total zinc (14.31 mg / kg) in the HZn group compared to MZn (11.55 mg / kg; $p < 0.05$) and LZn (12.45 mg / kg; $p < 0.1$) was detected in mesenteric lymph nodes after one week of feeding the zinc diets. We observed that feeding the HZn diet for one week increased the level of activated T-helper cells ($CD4^+$, $CD8\alpha^{dim}$) as compared to the MZn- and LZn-group ($p < 0.05$). In addition, we observed higher transcript amounts of *IFNγ* and *TBET* in the HZn-group compared to the MZn- and LZn-group ($p < 0.05$). Gene set enrichment analysis of RNA-Sequencing data, revealed an overrepresentation of genes associated with "Cytokine signaling in immune system" after one week feeding the HZn diet. Remarkably, feeding of a very high zinc dosage led to a switch in the immune response, after two weeks feeding. We detected higher relative cell counts of $CD4^+CD25^{high}$ regulatory T-helper cells ($p < 0.05$), and a higher expression of *FOXP3* transcripts ($p < 0.05$). After four weeks feeding the high zinc level diet the relative $CD4^+$ T-cell count ($p < 0.05$) and the relative $CD8\beta^+$ T-cell count ($p < 0.1$) were reduced compared to the MZn-group.

Conclusion: We hypothesize that after one week the cellular T-helper 1 response is switched on. After two weeks it is switched off leading to decreased numbers of T-cells. Therefore, our research suggests that short term feeding (one to two weeks) of high levels of zinc at the critical time point of weaning has an immune activating effect on the adaptive T-cell response, and could provide an alternative to reduce the incidence of post weaning diarrhea in pig husbandry. However, our findings indicate that long term feeding (two to four weeks) of high levels of zinc seems to have an immune suppressive effect in post weaned piglets, and as such could potentially negate the benefits of zinc supplements in post weaned piglets.

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6.

Alteration of Ca transporting structures in small intestine and egg shell gland of phylogenetically divergent chicken lines after dietary calcium depletion

Beeinflussung der Ca-transportierenden Strukturen im Dünndarm und in der Eischalendrüse bei phylogenetisch unterschiedlichen Hühnerlinien durch eine Ca-Restriktion

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In high performing laying hens, approximately 2 g Ca are needed for egg shell calcification daily. To meet this high demand, hens rely on a complex interplay of intestinal absorption and mobilization of Ca from the skeleton. To investigate whether performance level has affected the capacity to adapt to changes in Ca supply, this study was performed using four different purebred layer lines that varied in laying performance and phylogenetic origin. In total, 132 hens, 33 of each line, were allocated to two different feeding regimes. The diets applied to the hens ad libitum can be classified as adequate (Ca+; 4.3%) or deficient (Ca-; 1.1%). With beginning of the 31st week of age, the control groups (11 hens per line) were fed the Ca+ diet over the entire 21-week experiment. The test groups (22 hens per line) received a Ca- diet three times for a 3-week-period each. The first and the second Ca- period were followed by 6-week recovery periods (Ca+ diet). After the third Ca- period (52nd week of age), the animals were sacrificed. Tissue samples of duodenum, ileum and egg shell gland were analyzed for the expression of mRNA and protein of $Calbindin_{28kD}$ and PMCA, transport proteins mediating active, transepithelial Ca transport, by means of Real-time PCR and Western Blot. In case of PCR results, an ANOVA F-Test was conducted. Afterwards a Tukey's HSD-Test was performed for multiple comparisons of means. Western Blot results were analyzed by a t-test. Statistical significance was set at $p<0.05$. Analysis of variance for Calbindin revealed a significant effect of diet in duodenum ($p < 0.0001$) and ileum ($p < 0.0001$) and a significant influence of line and diet in ESG ($p < 0.0082$). In case of PMCA, a significant effect of diet in duodenum ($p < 0.0001$) and an effect of diet ($p < 0.0047$) and line ($p < 0.0393$) in ileum was detected. The low performing brown-layer line L68 showed an increase of transcripts of $Calbindin_{28kD}$ in the small intestine (duodenum and ileum) and an increase of protein in ileum ($p < 0.0094$). A stimulation of intestinal PMCA expression could be observed, too, but only in respect of BLA (high performing brown-layer) hens, it was significant in the duodenum on both, mRNA- and protein-level ($p < 0.0139$). In the high performing white-layer line WLA, we only observed an increase of protein expression of $Calbindin_{28kD}$ in the small intestine (duodenum ($p < 0.0120$) and ileum($p < 0.0102$). An effect on expression of $Calbindin_{28kD}$ in the egg shell gland was exclusively found in L68 hens ($p < 0.0194$). Taken together, L68 hens showed the best adaptation to a reduced Ca supply by increasing the Ca-transporting structures, while WLA hens seem to adapt least.

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7.

In situ ruminal degradation of phytate from single and compound feeds for dairy cows

Ruminaler in situ-Abbau von Phytat aus Einzelfuttermitteln und daraus hergestellten Mischfuttermitteln

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The ruminal degradation of phytate ($InsP_6$) varies between different feedstuffs (1). However, there is a lack of data of effective $InsP_6$ degradation for common feedstuffs used as concentrates in diets for dairy cows. Therefore, the aim of the study was to broaden the data basis of effective ruminal degradation (ED) of $InsP_6$ for different feedstuffs. Furthermore, we analysed and incubated two compound feeds and examined if additivity of $InsP_6$ concentration and ED of $InsP_6$ of the single feeds is given in mixes of these feeds.
Methods: The in situ incubation followed recommendations (2). Nine single feeds (maize, wheat, barley, soybean meal (SBM), soybeans (SB), sunflower meal (SFM), faba beans (FB), rapeseed meal (RSM), and dried distillers grains with solubles (DDGS)) as well as two mixtures consisting of different amounts of aforementioned single feeds and containing 22 and 24 % crude protein in dry matter were investigated (22_M; 24_M). The mixtures were prepared with a commercial feed mill (RKW Kehl, Germany) under industrial conditions without pelleting. Feeds were ground and incubated in the rumen of three fistulated Jersey cows for 2, 4, 8, 16, 24, 48, and 72 h, providing three replicates per time point of each feed. The washout fraction of the concentrates (incubation time 0 h) was determined by analysing the bag residues after washing in a commercial washing machine. Mixture 22_M consisted of 10% maize, 46% barley, 5% SBM, 18% SB, 16% FB, and 5% DDGS, mixture 24_M of 32% maize, 12% wheat, 8% SBM, 10% SFM, 16% FB, 17% RSM, and 5% DDGS. The ED of $InsP_6$ was calculated at a ruminal outflow rate of 8%/h (ED8) for all feeds. Additionally, the concentration and ED of $InsP_6$ was calculated for both mixtures from the single feeds to assess the additivity of $InsP_6$ concentration and degradation. The ED values were analysed statistically with the PROC MIXED feature of SAS (9.4) in a one-factorial approach with animal as random effect.
Results: The $InsP_6$ concentrations in the single feeds (per kg DM) were as follows: maize: 7,0 g, wheat: 8,2 g, barley: 6,3 g, SBM: 17,0 g, SB: 14,4 g, SFM: 32,9 g, FB: 14,4 g, RSM: 23,5 g, DDGS: 4,6 g, 22_M: 8,8 g, 24_M: 14,4 g. Calculating the $InsP_6$ concentrations for 22_M and 24_M from the concentrations of the single feeds led to 9,6 and 14,5 g/kg DM, respectively. ED8 differed significantly between feedstuffs and was highest for FB (92%), maize (90%), and DDGS (89%), followed by SB (85%), wheat (81%), and barley (79%). The lowest ED8 was determined for SBM (67%), SFM (65%), and RSM (46%). For 22_M and 24_M, ED8 was 83 and 74%, respectively. Calculating ED8 of $InsP_6$ from the values of the single feeds resulted in 83% for 22_M and 69% for 24_M.
Conclusions: The ED values of $InsP_6$ differed largely between the examined feeds. But even at a passage rate of 8%/h, the $InsP_6$ degradation was in the range of 65% or distinctly above for all feeds, except for RSM, proving the high potential of rumen microorganisms for $InsP_6$ hydrolysis. For processed protein feeds such as SBM and RSM, however, it has been shown that processing conditions influence the ED of $InsP_6$ to a large extent and, thus, values cannot necessarily be transferred from one examined meal to another. Additivity was given for the $InsP_6$ concentrations in both mixtures when calculated from the values of the single feeds. Furthermore, calculating ED8 for 22_M resulted in identical values when incubated or calculated from single feeds. Although ED8 for 24_M was underestimated by 5% when calculated from single feeds, it gave a sufficiently approximate value indicating that the concentration as well as the ED of $InsP_6$ for compound feeds can be calculated from the respective values of their ingredients.

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8.

Differential expression of genes associated with Mg^{2+} homeostasis in dairy cows with high and low back fat mobilization

Differentielle Expression von mit der Mg^{2+} Homöostase assoziierten Genen in Milchkühen mit hoher und niedriger Rückenfettmobilisierung

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During the transitional period, the physiological state of dairy cows changes dramatically. The demand for energy for lactation is higher than the feed intake. This imbalance between energy uptake and energy demand inevitably leads to a negative energy balance (NEB) (1). In order to cover the needs, mobilization of body fat increases dramatically with parallel increases in the production of ketone bodies, predisposing these animals for ketosis and fatty liver (2). Magnesium is an essential nutrient and plays a crucialrole in many enzymatic processes. The aim of this study was to investigate the expression of genes associated with Mg^{2+}transport, namely SLC41A1, SLC41A2, SLC41A3, CNNM2, NIPA1, MagT1, MRS2 and TRPM6/7, in different tissues of high- and low-mobilizing cows in order to investigate the influence, relation and dysregulation of the intracellular magnesium homeostasis on metabolic pathways associated with lipomobilization. **Methods:** Samples from kidney, liver, and leucocytes were collected from seven high-mobilizing and seven low-mobilizing healthy cows. The animals were grouped according to the degree of back fat reduction between calving and the time of slaughter. The high mobilizing group consisted of animals with a back fat reduction of -10 to -19 mm, whereas the low mobilizing group comprised animals with a reduction between -2 and -8 mm. Total RNA was isolated by a standard spin column-based method and samples with adequate quality (RIN higher than 8) were reverse transcribed to cDNA. SYBR-Green-based qPCR was performed in triplicate, RPS9, RPS19 and GusB served as reference genes and were used for normalization. Relative expression was calculated using the ΔΔCT method. Calibrated normalized relative quantity (CNRQ) values were used for statistical analysis by Student's t-test. *P*values of <0.05 were considered to be statistically significant. **Results:** The expression of TRPM7 showed the most prominent differences. A significantly higher expression of TRPM7 expression was found in kidney (CNRQ: 2.06 ± 0.28vs. 1.11± 0.22 , $P = 0.024$), liver (CNRQ: 2.22 ± 0.21 vs. 1.09± 0.19, $P = 0.002$) and leukocytes (CNRQ: 5.42 ± 0.62 vs. 2.99± 0.63, $P = 0.017$) of the low vs. high-mobilizing animals. Furthermore, higher expression of MagT1 (CNRQ: 0.65 ± 0.06 vs. 0.39 ± 0.006 , $P = 0.012$) and NIPA1 (CNRQ: 1.45 ± 0.09 vs. 1.04± 0.14, $P = 0.031$) were observed in kidney samples of low vs. high-mobilizing cows. Also a stronger expression of MRS2 was observed in the leukocytes samples of the low-mobilizing group (CNRQ: 3.9 ± 0.37vs. 2.24± 0.47, $P = 0.015$). Interestingly, expression of TRPM6 in liver samples of low-mobilizing cows was lower than in high-mobilizing group (CNRQ: 0.49 ± 0.09 vs. 1.3 ± 0.42,$P = 0.038$). No significant differences were observed for the expression of the other tested genes. **Conclusion:** Since TRPM7 represents the main entry mechanism for magnesium into cells, the observed higher expression of this gene in low-mobilizing cows might indicate a better supply of various tissues with Mg^{2+}in these animals. Moreover, the higher expression of NIPA1 and MagT1 in kidneys of low-mobilizing animals could support a more efficient reabsorption of $Mg^{2+}$and may contribute to a better whole body Mg^{2+} status. TRPM6 has been shown to be upregulated in response to Mg^{2+} deficiency. The significantly higher expression of TRPM6 in the liver of high-mobilizing cows might be a further indicator for an insufficient Mg^{2+}supply in these animals. Insulin insensitivity during the transitional period is a physiologically process. However, an exaggeration in this insensitivity predisposes cows to metabolic and inflammatory diseases (1). Given the central role Mg^{2+} in energy metabolism and that the ion is indispensable for the efficacy of insulin and glucagon signaling, a better Mg^{2+} status might therefore counteract excessive lipomobilization.

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This work is supported by an Elsa-Neumann-stipend to Sandra Jurek.

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9.

Effects of different dietary concentrations of copper sulfate and copper glycinate without or with formic acid *on performance and copper metabolism in* piglets

Wirksamkeit verschiedener Konzentrationen von Kupfersulfat und -glycinat ohne und mit Ameisensäure auf die Leistung und den Kupferstoffwechsel bei Absetzferkeln

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The trace element copper plays an important role in piglet feeding because of its beneficial effect on the digestive function and animal performance. The EU has set the maximum copper content to 170 mg/kg piglet feed for a maximum of 12 weeks of life. EFSA recommended that the copper content in complete feed for piglets should not exceed 25 mg/kg. The objective of the trial was to study the effects of copper sulphate at different concentrations including the currently authorised level (5, 25, 170 ppm) in post-weaning piglets in comparison to Cu-glycinate at (25 ppm) with addition of inorganic or organic sources of Fe, Mn, and Zn at recommended dose levels (Fe: 70 ppm; Mn: 20 ppm; Zn: 60 ppm) without or with formic acid from d 25 to d 66 of age (42-d feeding period).

Methods: The experimental design was a randomized block with eight treatments and 6 replicates of ten post-weaning piglets per experimental unit (pen). The 42-d feeding period was divided into a starter period (25 to 38 days of age) and a grower period (from 39 to 66 days of age), respectively. The starter and grower diets for post-weaning piglets were produced on barley, corn, soybean meal and wheat and followed the recommendations of the German Society for Nutrition Physiology for pigs (GfE 2006) except copper. Enzymes or other zootechnical feed additives were not added. A total of 8 complete starter and grower diets was produced: 1-3: Fe: 70, Mn: 20, Zn: 60, Cu: 5/25/170 mg/kg as sulfates; 4: Fe, Mn, Zn as sulfates, Cu: 25 mg/kg as glycinate; 5: Fe: 70, Mn: 20, Zn: 60, Cu: 25 all as glycinates; 6: Fe: 70, Mn: 20, Zn: 60, Cu: 25 mg/kg as sulfates plus 0.8 g/kg formic acid; 7: Fe 70, Mn: 20, Zn: 60 as sulfates, Cu: 25 mg/kg as glycinate plus 0.8 g/kg formic acid; 8: Fe: 70, Mn: 20, Zn: 60, Cu: 25 all as glycinates plus 0.8 g/kg formic acid. Piglets were monitored for any abnormalities, abnormal behaviour, and clinical signs of sickness daily. Feed conversion ratio (FCR, feed: gain) was calculated on a weekly basis. For measuring the apparent ileal and total tract digestibility of trace elements titanium dioxide was added as marker to the diets. Six piglets from each treatment with body weights closest to the average of their treatment groups were used for measurements of tibia and liver mineralization. Data were analyzed by one-way ANOVA, comparisons between treatment groups were made by Tukey's test ($P \leq 0.05$).

Results: Animal health was not affected by the different diets. Highest body weight gain was observed in the group fed 170 mg Cu/kg as copper sulphate ($p = 0.008$). When using chelated trace minerals with copper at the reduced level, the cumulative body weight was numerically enhanced compared to the inorganic sources at similar dose levels. In combination with formic acid, performance numerically increased in comparison to organic bond trace minerals without acid. Feed conversion tended to be improved in the group fed 170 mg Cu/kg as copper sulphate ($p = 0.052$). Apparent total digestibility of copper was affected by dietary copper sources and levels ($p < 0.001$), lowest values (6.4%) were observed with the diet containing 170 mg Cu/kg as copper sulphate, highest apparent digestibilities were found with copper glycinate (24.1 – 26.2%), intermediate values were found with 5 and 25 mg Cu/kg as copper sulphate (12.4 – 14.3%). The inclusion of formic acid had no significant effect on the total tract digestibilities. The apparent digestibilities of the other trace elements were not different between the groups. Trace element concentrations in the tibia were not affected by the dietary treatments; in the liver, the highest copper concentrations were determined in piglets fed the diet containing 170 mg Cu/kg as copper sulphate ($p < 0.001$).

Conclusion: The data indicate that dietary copper levels in piglets *can be reduced without adverse effects on health and performance.*

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10.

Copper deficiency in fallow deers (*Dama dama*) from farms in Lower Saxony?

Kupfermangel bei Damwild (Dama dama) in niedersächsischen Gatterwildbeständen?

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After a number of cases of paresis/paralysis of the hind limbs occurred in calves from several fallow deer (*Dama dama*) keeping farms in Lower Saxony, Germany, the involved veterinarian suspected a copper (Cu) deficiency. The following study was performed to confirm or to rule out this suspicion and moreover to gather quantitative data regarding the current state of copper supply of fallow deers kept in enclosures in northern Germany.

Methods: A total of five farms were included in the study. Questionnaires were sent to the owners in order to receive detailed information regarding the use of Cu containing mineral supplements and fertilizers as well as the deworming management on the respective farms. Furthermore tissue and organ samples (blood, liver and muscle) from several clinically inconspicuous fallow deers of each farm were taken at slaughter. In total 20 animals were included in the study (17 of them were younger than two years), number of males and females were 13 and seven, respectively.

Results and discussion: From the four farms that replied to the questionnaire, only one offered a mineral supplement containing Cu and none of the farms used fertilizers with trace elements. Deworming was not conducted for at least one year on any of the farms. On average the Cu concentration in serum was 61.4 ± 23.8 µg/dl (males [n = 13]: 58.5 25.6 µg/dl, females [n = 5]: 69.0 ± 18.8 µg/dl). In liver (n = 19) and muscle (n = 18) samples on average 11.3 ± 4.01 mg and 3.94 ± 0.726 mg Cu per kg DM were found, respectively. Compared to literature data (1) the analysed values varied at a quite low level. As sandy soils in northern parts of Germany are known for their low copper level an insufficient supply seems likely. Among factors influencing Cu supply parasite infection might also be associated with changed Cu concentrations in blood and liver of fallow deers (2). Although the rather low number of animals in the present study does not allow a definitive statement on this, the results seem to support the findings of VENGUŠT a. VENGUŠT (3) that Cu content in liver is higher in female fallow deers compared to male ones. In the present study females on average had higher Cu contents in liver (males [n = 13]: 10.3 ± 2.83 mg/kg DM, females [n = 6]: 13.5 ± 5.50 mg/kg DM) and muscle (males [n = 13]: 3.78 ± 0.487 mg/kg DM, females [n = 5]: 4.34 ± 1.12 mg/kg DM).

Conclusion: Compared to literature references results of the present study indicate a rather low Cu supply in deers of all farms. Taken into account that only one farm fed a Cu containing mineral supplement and trace elements were not used for fertilization of grassland on any of the farms, an inadequate Cu supply seems likely but other reasons for these findings cannot be ruled out.

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11.

Lameness in fattening turkeys due to mineral deficiencies in a complete compound feed – a case report

Lahmheiten bei Mastputen aufgrund eines Mineralstoffmangels im Alleinfutter – ein Fallbericht

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Lameness is a frequent problem in poultry farms concerning health and welfare of the animals and can be caused by infectious or non-infectious agents. In addition, economic losses are seen subsequently, so that the etiology should be clarified as fast as possible. Beside dietary contents of calcium and phosphorus, the P availability (phytate phosphorus, phytases) and the vitamin D_3 content are of special interest, without neglecting trace elements like copper, zinc or manganese (1).

Methods: In a fattening turkey farm with 10,000 hens, animals suffered from an impaired walking ability at the age of three weeks (complete compound feed: phase 1). The veterinarian observed lameness in the animals and found very flexible bones during dissection of affected animals ("breaking test" of bones). As there were no signs of infections obvious, the veterinarian decided to administer complementary feeds rich in phosphorus and vitamin D_3 (77 g P/L, 24 g Ca/L, 500,000,000 I.E. Vit. D_3/L) via the drinking water due to the suspicion of an insufficient mineral supply. The animals recovered during the next weeks (phase 2), but at the age of six weeks (phase 3) the symptoms reemerged in an even more severe form. Again there were no hints on infectious processes, so the feed batch was replaced immediately, the named complementary feeds were administered via drinking water and a representative feed sample was taken for chemical analyses according to official VDLUFA Methods: The complete compound feed contained monocalcium phosphate and wheat bran as main P sources and a phytase was added (750 FTU 6-Phytase EC3.1.3.26(4a19)).

Results: The chemical analysis revealed a crude ash content of 45.7 g/kg (as fed; DM content: 892 g/kg), a calcium content of 2.62 g/kg and a phosphorus content of 5.39 g/kg, which were both below official recommendations (2). Furthermore the inverse Ca:P ratio is worth to be mentioned (3). Other nutrients as crude protein, crude fat, crude fibre, sodium vitamin D_3, phytase activity and the energy content were in accordance with the declaration. The exchange of the complete compound feed resulted more or less in a recovery of the herd, but in some individuals lameness and a variance in growth was still obvious after further three weeks. The administered complementary feed contained an inverse Ca:P-ratio, so that it was not suitable in this case.

Conclusions: The case report illustrates the importance of an immediate chemical analysis of the diet in cases of lameness in poultry herds. Although the usage of complementary feeds via drinking water is a possibility for administering lacking nutrients as a fast reaction, the type and extent of the deficiency have to be known. Besides, infections have always to be considered as a differential diagnosis.

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12.

Influence of feed fermentation on phytate content and phosphorus digestibility in fattening pigs fed a liquid diet based on rye and rapeseed extracted meal

Einfluss der Fermentation eines Flüssigfutters (basierend auf Roggen und Rapsextraktionsschrot) auf Phytingehalte und Phosphor-Verdaulichkeit bei jungen Mastschweinen

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Introduction: Fermentation of liquid feed (~ for 24 h) is an increasingly popular feeding concept for pigs [1]. In 'controlled' fermentation a starter culture is added at fermentation's start ('uncontrolled' fermentation: no starter culture). Aim of the study was to compare phytate-contents before, after uncontrolled as well as after controlled fermentation and to prove the effects of controlled fermentation on P digestibility in young fattening pigs without adding phytase, but using high contents of rye (supposed: high intrinsic phytase activity).
Materials and methods: The fermented liquid feed (FLF) was produced in two identical Mini-Fermenters, filled daily and fermented for 24 h. In controlled fermentation a freeze-dried, granulated starter culture (SchaumaLac Feed Protect XP G, H. Wilhelm Schaumann GmbH, Germany) was added at the fermentation start in a dosage of 2 x 10^5 cfu/g liquid diet. The Mini-Fermenter was closed after filling and the suspension was stirred therein every hour for 60 seconds at 900 rpm (temperature 34 – 38 °C). The liquid diet consisted (% of DM) of rye 48.2, rapeseed extracted meal 29.4, wheat 9.80 and barley 9.84 (feed water : water ratio 1 : 3.2), without any phytase addition. Contents of total-P (colorimetric estimation), phytate-P (IP 6-P) and degradation products IP 6, IP 4, and IP 3 were analysed (ion chromatography) at three times: Before, after uncontrolled and after controlled fermentation. In a feeding trial 10 growing pigs (bw: 19.9 ± 1.55 kg) were randomly divided in two groups, individually housed and fed *ad libitum* over 4 weeks. Group 1 received the diet in native form (= unfermented / mixed immediately before feeding with water), whereas group 2 was fed the same diet after 24 h of 'controlled' fermentation. A mineral supplement (whole diet: inorganic P: ~ 1 g/kg DM, but no phytase) was added just before feeding. In the 2nd week (after adaptation period of 7 d) the faeces were collected individually and completely for a period of 7 days to determine the apparent P digestibility. Statistical analyses were done by using SAS® software (Mean, SD and t-test).
Results: Before fermentation the total-P content in the FLF varied at 5.44 ± 0.164 g/kg DM. Nearly 40 % of P were bound to IP 6 (2.04 ± 0.404 g/kg DM). After fermentation phytate content strongly decreased. After 24 h of uncontrolled *and* controlled fermentation the IP 6 content varied below the limit of determination (IP 6: < 0.6 g/kg DM). After controlled fermentation even IP 5-P, IP 4-P and IP 3-P were not quantifiable, whereas after uncontrolled fermentation still low contents of IP 3-P were present (0.201 ± 0.088 g/kg DMà 3 – 4 % of total-P). In the feeding trial the P digestibility was significantly enhanced by feeding FLF: 72.8 ± 3.02 % vs. 54.2 ± 4.94 % in pigs fed the fermented and unfermented diet, respectively.
Conclusion: Storing feed (based on rye and rapeseed extracted meal) in a liquid form combined with fermentation resulted in marked effects on phytate content and its degradation products as well as on P digestibility. Without adding any starter culture the phytate degradation was incomplete (IP 3-P detectable), whereas after controlled fermentation IP 3-P was completely degraded. This study demonstrates the unique potential of FLF based on rye (= high intrinsic phytase activity) to maximize P digestibility in pigs even if high proportions of P were derived from rapeseed extracted meal.

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13.

What is the impact of organic acids, zinc and copper on the transfer of extended-spectrum beta-lactamases carrying?

Welchen Einfluss haben organische Säuren, Zink und Kupfer auf den Transfer von Extended-Spectrum Beta-Lactamasen tragenden Plasmiden?

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Introduction: The genes encoding for extended-spectrum ß-lactamases (ESBL) are frequently located on plasmids. These plasmids can be transferred from animal adapted to human adapted bacteria as well as from non-pathogenic species to potential pathogens. Nutritional modifications, such as probiotic feed additives, have been successfully used to reduce the amount and transfer of ESBL-producing bacteria (1, 2). The question remains, how these methods impact the transfer of the ESBL genes.

Methods: Equal amounts and concentrations of an ESBL-producing *Escherichia coli* donor strain and a *Salmonella* Typhimurium recipient strain were inoculated in Müller Hinton 2 Broth containing different levels of lactate, propionate, acetate, butyrate, copper or zinc. The solutions were incubated aerobically at 37°C for 4 hours and thereafter serially diluted and spread on MacConkey agar with and without antibiotics. Donor, recipient and total bacterial counts were obtained from the MacConkey agar without antibiotics. Transconjugant counts were estimated from the MacConkey agar plates containing cefotaxime and sulfamethoxazole/trimethoprim. Conjugation frequency was calculated as transconjugants/donor, transconjugants/recipient and transconjugants/total bacterial count. Statistics were calculated with the software IBM SPSS (Version 22), using the non-parametric Kruskal-Wallis test and pairwise comparison. Differences were considered significant if $p<0.05$.

Results: The presence of lactate and butyrate had no significant impact on the conjugation frequency. A reduction of the conjugation frequency by 0.5 log units was observed in the presence of acetate when referred to the donor and total bacterial count. High levels of propionate decreased the conjugation frequency calculated on donor, recipient or total bacterial counts with 1.7-2.0 log. A concentration depending impact was observed if zinc was added to the medium and the conjugation frequency was calculated on donor or recipient counts, reducing the transfer with up to 0.8 log units. If calculated by total bacterial counts, the conjugation frequency showed a similar trend. A strong correlation between increasing copper concentrations and decreasing conjugation frequencies was observed for all three methods of calculation. The highest observed influence on conjugation with a reduction exceeding 3 log was observed in the copper setup.

Conclusions: Nutritional compounds have an impact on the transfer of ESBL-carrying plasmids. The highest impact was observed in the presence of copper.

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14.

AMP-activated protein kinase as mediator of short-term adaptation to hypoxia in lagomorph jejunum epithelium

AMP-aktivierte Proteinkinase als Mediator der kurzfristigen Anpassung an Hypoxie im lagomorphen Jejunumepithel

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AMP-activated protein kinase (AMPK) is known as an energy sensor regulating the cellular metabolism according to the intracellular ATP levels [1]. A major target of AMPK is the downregulation of energy consuming, i.e. primary or secondary active transport mechanisms in order to save valuable energy. However, there is increasing evidence, that it is also involved in the cellular adaptation to other extreme situations like inflammation and hypoxia, irrespective of cellular energy levels [2]. We hypothesized that its influence on protein activity could be an important part of the quick adaptation of intestinal epithelial cells to hypoxia. Therefore, we investigated the effect of hypoxia on the activation of AMPK and the secondary active transport of glucose into the enterocytes via sodium coupled transport (SGLT1).

Methods: Lagomorph jejunal epithelia were incubated in Ussing chambers under short circuit conditions. Hypoxia was simulated by gassing with 99% N_2 + 1% O_2 ('hypoxia') instead of 100% O_2 (control). The activity of SGLT1 was assessed by measuring the increase of short circuit-current (I_{sc}) after addition of 2 mM glucose to the mucosal buffer solution. The activation of AMPK, i.e. its phosphorylation, was assessed by Western Blot analysis. Additionally, the epithelia were preincubated with its inhibitor compound C and its agonist AICAR, respectively. After preincubation, the influence of AMPK activation on SGLT1 was measured as the sensitivity of I_{sc} to the SGLT1 inhibitor phlorizin. The expression of SGLT1 in the apical membrane was assessed by Western Blot analysis of apical membrane vesicles.

Results: We observed a decreased electrogenic response to mucosal addition of glucose after prolonged hypoxia compared to control conditions (one-way repeated measures ANOVA, $p<0.01$, $N = 7$ animals). The response could be restored by preincubation with the AMPK-antagonist compound C (two-way repeated measures ANOVA, $p<0.05$, $N = 9$). Western Blot studies showed an increased phosphorylation of AMPK (pAMPK) under hypoxia as well as incubation with AMPK-agonists but a significant decrease in pAMPK under hypoxia after incubation with compound C (paired t-test, $p < 0.05$, $N = 6$). The expression of SGLT1 in the apical membrane was not changed under hypoxia compared to control conditions (preliminary results, paired t-test, $N = 3$).

Conclusions: The activity of SGLT1 is decreased under hypoxic conditions AMPK-dependently (see also [3]). This does not appear to be related to the membrane localisation of SGLT1 but might be due to an inactivation of the transport protein by phosphorylation.

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This study is supported by the DFG (DE 2489/1-1).

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15.

Use of milk recording data for characterisation of dairy cow supply situation

Nutzung von Milchkontrolldaten zur Charakterisierung der Versorgungslage von Milchkühen

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For most of the German dairy farms data from standard milk recording are available and can be used cost-efficiently for herd management support. For characterisation of lactating dairy cows supply with feed energy and protein a nine-field table is prevalent that contains milk protein (P [%]) and milk urea (U [mg/l]) level. This schematic is based on references from the early nineties (1). In the last 30 years milk yield per cow increased sizeable and the old scheme conclusions do often not match with other animal related parameters now. But if those evaluations are used to scale animal welfare and are integrated into milk payoff, there is a need to have more accurate information from the milk ingredients. Additionally with regard to environmental effects, an overflow with feed protein should be prevented.

Methods: For this research a dataset of 7,284,904 milk test day data from 952,603 cows of 13 different breeds, housed at 8,954 dairy farms in Germany and Luxembourg was used; Angler and Jersey cows are not included in this abstract. The dataset was kindly provided by all German and Luxembourgian milk recording associations and represents about 20 % of all recordings of the year 2015. Plausibility was checked according to the official guidelines. Days in milk (DIM) and fat protein ratio (FPR) were calculated for each record. To show interactions between fat (F [%]), P, FPR and U data were analysed using SAS, Version 9.4 calculating linear regressions and Pearson coefficients of correlation. To investigate the impact of U on milk yield, data were classified into steps of 50 mg U per litre.

Results: The higher the milk yield the higher the degree of dilution concerning F and P. But the ratio of F and P is nearly independent on milk yield. This is demonstrated by the regression and determination coefficients (r^2) of the relations of F, P and FPR respectively with milk yield (-0.0325 vs. -0.0226 vs. -0.00164 kg milk/day; $r^2 = 0.157$ vs. 0.255 vs. 0.0058). While correlation between F and P is r = 0.485, the FPR is more influenced by F (r = +0.754) and less by P (r = -0.198). This connection is more pronounced (F: r = +0.841; P: r = -0.271) within the first 60 DIM. The FPR of animals with +25 % or +10 % best performance in the first 60 days of lactation is 1.27 and 1.26, respectively. There was nearly no relationship between U and F, P or FPR. The linear regression line over all data has just a very small increase for the dependence of milk yield and U (+0.0049 kg milk/mg U; $r^2 = 0.0015$), but regarding the classified data an increase of yield could be observed for the classes < 100, 100 to 149 and 150 to 199 mg U /l milk (regression coefficients: +0.03941, +0.03745 and +0.02225 kg milk/mg U; $r^2 = 0.0076$, 0.0034 and 0.0012). For cows with 200 - 249 mg U there is virtually no more increase and a zero coefficient of determination (+0.00611 kg milk/mg U; $r^2 = 0.0001$) and in the further higher U classes there is even a decrease in milk yield (e.g. class 250 - 299: -0.00605 kg milk/mg U; $r^2 = 0.0001$).

Conclusions: The FPR is a better indicator for feed energy supply than milk protein, because it is less dependent on milk yield. FPR is positively affected by F and less negatively affected by P particularly in early lactation. Therefore, it is an appropriate indicator for energy supply especially for this period. The U classified data showed a turn in the algebraic sign for regression coefficients between milk yield and milk urea. The results suggest a further development of evaluation tools for characterisation of feeding: a six-field table containing FPR as an indicator for energy supply and milk urea as an indicator for supply with feed protein and indirectly with feed energy. As a threshold for a lack of feed energy we recommend a FPR of more than 1.4, according to internationally used schemes. For milk urea there should be a lower limit at 150 mg/l and an upper limit at 250 mg/l. There is no nutritional need for higher urea (2)(3).

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16.

Can chewing behavior improve estimation of dry matter intake of individual dairy cows?

Kann die Schätzung der Futteraufnahme von einzelnen Milchkühen anhand des individuellen Kauverhaltens verbessert werden?

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Dry matter intake (DMI) is one of the most important nutritive factors affecting productive performance of high-yielding dairy cows. However, accurate estimation of DMI of individual animals is still challenging under experimental in-house conditions and even more so under commercial on-farm conditions. With recent advances in automated systems to continuously record chewing behavior, the use of behavioral parameters such as feeding or rumination time has been suggested to improve the precision of models to predict DMI (e.g. 1, 2, 3). Aim of this study was therefore to test behavioral parameters recorded by automatic chewing sensors for their ability to improve the precision of DMI estimates (kg/d) of individual dairy cows. **Methods:** In four experimental runs, in total 29 primi- and multiparous lactating German Holstein cows were used with a mean body weight of 668 kg (standard deviation (SD) 64), a daily milk yield of 33.2 kg (SD 7.1), a range of 18 - 324 days in milk, and a mean DMI of 24.1 kg/d (SD 3.8, range 13.1 - 33.4). Cows were fed total mixed rations with maize silage and grass silage and were equipped with automatic chewing sensors (RumiWatch, Itin & Hoch, Liestal, Switzerland). Fresh matter intake was recorded by automatic weighing troughs (Waagen Döhrn, Wesel, Germany) and samples of offered diets analyzed for their chemical composition. Animals were milked twice daily and yield and composition of milk, body weight, head length, and mouth circumference of animals were recorded. In total, 220 daily records of chewing behavior, on average 5 d per cow (SD 2), were converted to 24-h-summaries. Data were analyzed by PROC CORR, PROC GLM, PROC GLM SELECT, and PROC MIXED options of SAS V9.4. The stepwise selection procedure was applied to identify variables that best predict DMI using adjusted r^2 and AIC as criteria. In case of covariance ($r>0.6$) among variables, only one of the correlated variables was selected. Selected variables were fed in a mixed model with cow as random effect. Marginal and conditional r^2 of the model were calculated with R (V3.5.1). **Results:** According to chewing sensors, cows spent on average 1,006 min/d (SD 84) chewing, with 441 min/d (SD 66) eating and 565 min/d (SD 57) ruminating. Number of eating chews correlated with fresh matter intake ($r = 0.35$; $p<.0001$), DMI ($r = 0.37$; $p<.0001$), and organic matter intake ($r = 0.47$; $p<.0001$). The product of mouth circumference as a proxy of bite volume and number of eating chews correlated to DMI ($r = 0.49$; $p<.0001$). Rumination chews (n/d and n/kg DMI) negatively correlated to body weight ($r = -0.37$ and $r = -0.49$; $p<.0001$) and age ($r = -0.58$; $p<.0001$; for n/kg DMI only), but did not correlate to DMI ($r = -0.07$; $p = 0.3$). Chewing rates (n/min) varied amongst individual animals. Rumination and eating rate (both in chews/min) could be explained by the class "cow" (PROC GLM, $r^2 = 0.96$ and $r^2 = 0.85$; $p<.0001$). Best predictors of DMI were milk protein yield (kg/d), eating chews (n/d), number of lactations, and mean metabolic body weight (kg) calculated from measurements of consecutive days. A mixed model containing these variables as fixed effects predicted DMI with a conditional $r^2_c = 0.89$ and a marginal $r^2_m = 0.68$. Removing the factor daily eating chews yielded an $r^2_c = 0.78$. A linear model without cow as random factor predicted DMI with $r^2 = 0.71$. Although adding mouth circumference improved the correlation of eating chews with DMI, this variable was not selected due to covariation with mean metabolic body weight ($r = 0.70$; $p<.0001$). **Conclusions:** Dairy cows exhibit individual chewing behavior in particular with respect to chewing rates, which correlate to covarying variables age and body weight. Coefficients of determination of the final model to predict DMI were high and improved significantly by adding number of daily eating chews. However, the potential of chewing behavior to predict DMI across a wider range of different diets should be further investigated.

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17.

Acceptance of elevated plains and their effects on litter quality, performance, and broiler behavior

Die Akzeptanz erhöhter Ebenen und ihr Einfluss auf die Einstreu und Leistung sowie das Verhalten bei Broilern

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To favor animal wellbeing and to avoid behavioral disorders there are widespread efforts regarding enrichment in broiler housing. Since history times it is well known that chicken prefer for nocturnal recovery elevated plains. Furthermore individual birds like to separate from the group for performing comfort behavior. Hypothesis of the present study was that there are various advantages for offering water and feed above elevated slatted plains to avoid excreta accumulation in the litter material beneath the feed and water lines; also splash water losses would not affect the litter directly, thus combining these approaches might result in reduced prevalence and intensity of footpad dermatitis. Finally birds´ performance is of economic relevance when measures of enrichment are implemented.

Methods: Three consecutive trials with 6800 broilers each were done: Trial 1 was focused on the `acceptance´ of elevated plains (n = 16; length: 600 cm; depth: 60 cm; height: 14 cm; 55.2 SI) by broilers without offering feed and water above them fitted. In a further trial the nipple drinkers' lines were installed above the elevated plains, covered with slatted floors; finally the feeders also were offered above these plains. As parameters which indicate the `acceptance´ the distribution of excreta within the barn (425 SI), and the dry matter content of litter material were measured. For comparison the litter moisture, a separate barn served as control one. Furthermore via video surveillance the behavior of broilers was monitored to generate data on the diurnal use and age effects. Finally daily gaines and feed conversion ratio were monitored.

Results: The evaluation of the video surveillance showed (1st trial, 2 -h intervals) that continuously a high number of broilers used the elevated plains (highest stocking values 04:00 / 10:00 am.). The body weight (abattoir data) of the experimental birds were lower in the three trials (control: $\bar{x}$ 2.08 kg; trial: $\bar{x}$ 1.96 kg; not significant), however the FCR was better (control: $\bar{x}$ 1.61^{a}; trial: $\bar{x}$ 1.43^{b}). In trial 1 and 2 the broilers´ foot pad health of the experimental barn were better ($\bar{x}$ score 0: control 91.2 %; trial 96.4 %), however the discard at the slaughterhouse were higher (control: $\bar{x}$ 1.28 %; trial: $\bar{x}$ 2.28 %) but both differences were not significant. Regarding the litter data in all 3 trials, the experimental barn had been drier and had a better distribution of excreta (control: $\bar{x}$ 12.2^{a}; trial: $\bar{x}$ 10.2^{b}; kg/m^2).

Conclusion: Based on the amounts of dry matter excreta and video surveillance, the acceptance of the elevated slatted plains was high. Offering feed and water above them fostered the use by the birds. The broiler´s preference of the elevated plains was so high that beneath the slatted floors the excreta (and other entries like "splash water losses") accumulated and thus reduced the litter moisture beside the plains and meanwhile improved footpad health. Broilers housed with elevated plains had in general a slightly lower body weight at trial´s end, however the better FCR. Nevertheless, caution is required. On the one hand "splash water losses" remain under the elevated levels but on the other hand "splash feed losses" are out of access to broilers as well, so this part of feed is lost.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE).

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18.

Identifying influences on the biological quality of drinking water for dairy cows

Bestimmung der Einflussfaktoren auf die biologische Tränkewasserqualität von Milchviehtränken

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Background: Water is the most important feed for animals and a very important factor concerning animal welfare, especially for livestock such as high-yielding dairy cows (1). In contrast to its importance, the legal regulations and official guidelines regarding the quality of animal's drinking water are rather unspecific. Especially influences on the biological water quality and associated risk factors are hardly known. The aim of this study was to evaluate the influence of several risk factors on the biological quality of water and to determine if different methods used in human hygiene are applicable to check the hygiene status of empty dairy troughs.

Methods: 105 dairy cattle troughs on 24 different dairy farms in Western Germany were evaluated. For each trough 25 different variable and permanent trough characteristics (material, volume, water temperature, e.g.) were recorded or inquired from the farmers. To evaluate the biological water quality, the trough water was tested on aerobic total viable count (TVC), coliform count (CC) and *Escherichia coli* (*E. coli*). Furthermore, the grown biofilm on the trough surface was analyzed on TVC, CC and *E. coli*. Rapid tests for protein as well as adenosine triphosphate (ATP) residues were used on the trough surface. Data were analyzed with Spearman rank correlations and linear models with post hoc Tukey for trough material, trough volume, water origin or water temperature with SAS 9.4 (2016).

Results & Discussion: The average TVC of the drinking water in the trough was 4.4 $\log_{10}$ cfu/ml. Coliforms were detected in 94.3% of all cattle troughs and *E. coli* in 48.6%, which verifies the urgent need to for routinely control and cleaning of troughs. TVC ($p<0.01$) and CC ($p<0.04$) in the water as well as TVC ($p<0.03$) and protein residues ($p<0.01$) on the trough surface were higher on farms using water from the communal water supply in comparison to farms using water from their own well. That might be explainable with correlations between the water origin and the trough cleaning interval ($R=0.31$, $p<0.01$) as well as the trough material ($R=0.49$, $p<0.001$). The most important trough characteristics influencing the biological water quality were water temperature and trough material. Higher water temperature led to higher TVC ($p<0.01$). Troughs made of cast iron (n=7) had higher TVC ($p<0.01$) and *E. coli* ($p<0.02$) in the water compared to stainless steel troughs (n=62) and higher TVC ($p=0.01$) compared to polyethylene troughs (n=36). Troughs with a volume of more than 120 l (n=31) had higher CC in water than troughs with small volumes below 5 l (n=24; $p<0.05$). Small volume troughs were only automatic drinking bowls, which had additionally best hygiene test results ($p<0.01$, respectively). CC ($R=0.46$; $p<0.001$) and *E. coli* ($R=0.31$; $p<0.01$) in the water were positively correlated with their equivalent in biofilm. Therefore, clean trough surfaces were essential for a good water quality or vice versa, supported by correlations of TVC as well as CC in water with hygiene tests (ATP and protein) on the cattle trough surfaces ($0.31>R>0.19$; $p<0.05$).

Conclusions: Troughs made of stainless steel seem to be favorable. Furthermore, cold water temperatures and automatic drinking bowls may enhance water quality, but conflict with animal welfare recommendations. Hygiene tests seem to be useful tools to directly check the hygiene status of troughs on farms. Nevertheless, more research is needed to develop trough cleaning strategies and optimal trough designs.

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19.

Serum methylmalonic acid concentrations in dairy cows with retained placenta, parturient paresis and healthy controls ante and post partum

Methylmalonsäure-Konzentrationen im Serum von Milchkühen mit Gebärparese, Retentio secundinarum und Kontrolltieren vor und nach der Geburt

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Due to its influence in the amino acid metabolism, nucleic acid synthesis and methyl-group transfer is cobalamin (i.e., vitamin B_{12}) an essential component for genomic stability. Assays for the measurement of serum cobalamin concentrations are available for cows and have been used extensively in the past (1), however those measurements do not accurately determine the cobalamin status. Concentrations of serum cobalamin are not a direct reflection of the intracellular cobalamin status. In human and veterinary medicine, decreased cobalamin concentrations are associated with increased methylmalonic acid (MMA) concentrations, a cobalamin-dependent metabolite, in serum (2). Increased serum MMA concentrations can result from a malfunction of methylmalonic acid-CoA mutase, a cobalamin-dependent enzyme. Changes in the serum MMA concentration reflect the intracellular availability of cobalamin and leads to a reduced supply of succinyl-CoA into the tricarboxylic acid cycle (i.e., Krebs cycle), which fuels cellular energy production. Thus, measurement of serum MMA concentrations may help to prevent cobalamin imbalances and metabolic diseases post partum in dairy cows. Currently, no studies are available that evaluate serum MMA concentrations in dairy cows. This study aimed to evaluate serum MMA concentrations in dairy cows with retained placenta, parturient paresis and healthy controls ante and post partum.

Methods: Serum samples from dairy cows with A) parturient paresis (n=13), B) retained placenta (n=11) and C) controls animals (n=13) from one farm were used. All cows had two to four lactations, same feed ration, an ante partum body condition score (BCS): 3.4 and no pre-existing conditions (e.g., mastitis or lameness). A collection of two serum samples were obtained on the farm at the same time seven days ante partum and a day post partum. Concentration of MMA (high-performance liquid chromatography / tandem mass spectrometry; limit of dection [LOD]: 34 nmol/L and assay varation: <3%) as well as beta-hydroxybutyrate (Hitachi 912 Automatic Analyzer; assay performance data: see manufacturer insrtuctions) and free fatty acid (Hitachi 912 Automatic Analyzer; see above), which both are commonly used biomarker for metabolic changes in dairy cows, were measured. Depending on the distribution of the data, a ANOVA / repeated measures ANOVA or Kruskall-Walles test / Friedmann test for multiple comparison of serum MMA concentrations among the three groups of dairy cows and a Pearson or Spearman rank sum correlation coefficient (ρ) for correlation analyses between serum MMA and beta-hydroxybutyrate as well as free fatty acid concentrations, were used.

Results: Serum MMA concentrations differed among the three groups of dairy cows 7 days ante partum (p=0.0128), with the highest MMA concentrations found in the parturient paresis group. The posttest showed a significant difference between parturient paresis and control group (p=0.0151), a trend between parturient paresis and retained placenta group (p=0.0593), and no difference between control and retained placenta group (p=0.8914). Serum MMA concentrations were significant lower post partum (1 day, BCS: 3,0; after treatment) when compared to ante partum (7 days; p=0.0031) in cows of the parturient paresis group. An association between MMA and free fatty acid concentrations was observed (ρ: -0.34 [95%CI: -0.50 to -0.17]; p=0.0001) but not between serum MMA and beta-hydroxybutyrate concentrations (p>0.05).

Conclusion: The results of this study suggest that the measurement of MMA, an indicator of the cobalamin status, in serum could be considered for early diagnosis of parturient paresis in dairy cows. However, further investigations are warranted to evaluate MMA concentrations in dairy cows with laminitis, ketosis and during lactation as higher amounts of cobalamin are needed for milk production.

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20.

Assessment of muscle mass in fattening bulls by means of body size measurements, bioelectrical impedance analysis and ultrasound measurements

Beurteilung der Muskelmasse bei Mastbullen mittels Körpergrößenmessungen, bioelektrischer Impedanzanalyse und Ultraschallmessungen

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The production of meat from ruminants is important to produce edible protein from plant material which cannot be used directly for human nutrition. Therefore the determination of muscle mass in beef cattle in vivo is important in animal nutrition experiments to calculate its accretion.

Methods: Thirty-two fattening bulls were slaughtered at different body weights (mean 609 kg, minimum 459 kg; maximum 785 kg). Before slaughter bioelectrical impedance analysis (electrode positions: shoulder blade region and hind limb of the animal) was conducted. A multifrequency impedance analyser (BIA 2000M, Data Input GmbH, Frankfurt/Main, Germany) was used. Resistance (R_S in ohms) and Reactance (X_C in ohms) were measured at frequencies at 1, 5, 50 and 100 kHz. Ultrasound measurements of muscle layers (*Musculus supraspinatus, M. infraspinatus, M. longissimus at vertebrae lumbales four, M. gluteus, M.biceps femoris, M. semimembranosus* as well as back fat thickness and fat layer on the 12th rib) were conducted with a Mindray M5 Vet (Mindray, Shenzhen, China) diagnostic ultrasound system equipped with a linear (6 MHz, Mindray 6LE5Vs) and a convex probe (3 MHz, Mindray 3C5s). Additionally body weight and body size measurements (hook height, withers height, hip width, heart girth, shoulder joint height and body length) were determined. During the slaughter process the carcass was divided longitudinally into two symmetric parts, weighed and stored at 4°C. The next day, carcass parts and the halves of the head were weighed once again for determination of water loss. Subcutaneous fat was dissected manually with a knife from the right carcass. The meat from the right carcass, right side of the head, right feet and the whole tail was separated from the bones manually with a knife, weighed and multiplied with two (except the meat from the tail) and summed up for calculation of the total skeletal muscle mass for the whole animal. An equation was developed for estimation of muscle mass by using the software R (Version 3.4.1). A stepwise forward regression with a step-by-step addition of variables according to Akaike´s Information Criterion (AIC) was computed. Extreme values were identified by Cook´s distance and eliminated at values higher than 0.15. An in-sample k-fold (k=5) cross-validation resampling process was conducted. In order to describe the accuracy of the equation, the coefficient of determination (R^2) and the root mean square error (RMSE) were calculated.

Results: The total skeletal muscle mass varied over a range from 95 kg to 185 kg with a mean of 145 kg and a standard deviation of ± 24 kg. Body weight, withers height, hip width, hook height, back fat thickness and shoulder joint height were selected in the model for estimating the amount of muscle mass with R^2 of 0.98 and RSME of 3.0 kg.

Conclusion: Under conditions of the present experiment for an estimate of muscle mass, the body weight is the most important variable to be included into the equation in addition to body size measurements. Furthermore, the inclusion of the back fat thickness implies an improvement of the estimation. The muscle layers measured with ultrasound and the bioelectric impedance measurements seem to produce no significant improvement in estimating muscle mass compared to body size measurements and back fat thickness alone in fattening bulls.

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21.

Time-dependent effect of intestinal microbial colonization patterns on concomitant immune development in neonatal piglets

Zeitabhängiger Einfluss der intestinalen mikrobiellen Besiedlungsmuster auf die gleichzeitige Entwicklung des Immunsystems in neugeborenen Ferkeln

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Question: Hyper-prolificacy in pigs increased the use of artificial rearing systems with formula feeding thereby influencing early-life gut colonization and immune development. We have recently shown that both formula and the artificial rearing environment delay gut microbial colonization and immune development (1,2). However, it could be speculated that such delayed conditions could be reversed within a specific "early-life time-frame". Thus, we studied the time-effect on gut colonization and immune reaction in neonatal piglets by using a back-fostering model.

Methods: A total of n=24 new-born piglets (male, female) were randomly allocated into four different groups (n=6 each; solely sow-reared (SOW), isolator-reared with formula for 3 days followed by back-fostering to the mother on day 3 (ISO3-SOW), isolator-reared with formula for 3 days followed by back-fostering to the mother on day 7 (ISO7-SOW), solely isolator-reared (ISO). Formula was based on skimmed milk powder and whey (22.6% CP, 20.0% EE, 46.0% lactose per kg DM) to get a comparable composition with commercial formulas. Fecal samples were taken from individual animals in days 1, 2, 3, 5, 7, 10 and 14 of life for DNA extraction and illumina sequencing of 16S rRNA gene amplicons. Sequences were analysed using the MG-RAST platform. At day 14 of life, peripheral blood mononuclear cell (PBMC) subsets were characterized by flow cytometry using the following antibodies: CD3, CD4, CD8, CD25, CD152mulg, CD172, SLAII, γδTCR1, Tbet, Foxp3. Analysis of bacteria and their relation to host-associated parameters was performed in R. Immunological data were evaluated by ANOVA in SPSS.

Results: Microbial diversity increased with age in all groups and no clear effect of neonatal environment on the total number of OTUs was observed. However, whereas all groups shared a relatively high number of similar OTUs during the first days of life, this number declined with age. Until the age of 14 days, microbial communities diverged between SOW and ISO groups with ISO3-SOW and ISO7-Sow groups showing intermediate patterns. Among the most abundant genera, *Clostridium, Lactobacillus* and *Bacteroides* became dominant in all groups finally reared with the mother, whereas less abundant genera such as *Fusobacterium, Collinsella, Ruminococcus* and *Veillonella* persisted in ISO piglets. Analysis of PBMCs did not show differences of T cell subsets (i.e. CD4+, CD8α+, CD4/CD8 dp, γδTCR1+), Natural Killer (NK) cells (i.e. CD335+/CD3-/CD172-) or antigen presenting (APC) cells (i.e. CD172+/CD3-) between the groups. However, lineage differentiation markers revealed a higher ($P<0.05$) abundance of Tbet expressing CD4+, and CD4/CD8 dp and γδTCR1+ cells in SOW as compared to ISO piglets. Similarly, a higher ($P<0.05$) abundance of CD152mulg+/SLAII+ among CD172+ was observed in SOW compared to ISO piglets, indicating a higher activation of APCs and Th1 polarization in the order SOW > ISO3-SOW > ISO7-SOW > ISO. Interestingly, a significantly higher abundance of NK cells with a CD152mulg+/SLAII+ phenotype was found in ISO piglets. This phenotype has been recently described in pigs (3) and might suggest a role of NK cells in antigen presentation during the early neonatal period and imbalanced microbial colonization patterns.

Conclusions: Isolator-rearing has strong influence on microbiota composition during the early neonatal period and can partially be reversed by moving piglets back into the mothers environment. The long term effects on immune system development needs to be further clarified – specifically the role of innate immune cells with APC function in ISO piglets is yet not clear.

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22.

Analyzing the effects of quercetin on porcine intestinal jejunum and follicle-associated epithelium of Peyer's patches

Analyse der Effekte von Quercetin auf porcines Jejunum und follikelassoziiertes Epithel von Peyer's Patches

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Outline: The jejunum consists of villous epithelium (VE) interspersed with follicle-associated epithelium (FAE) of Peyer's patches responsible for immune functions. Different barrier properties of VE and FAE have been characterized in detail recently (1). The plant flavonoid quercetin has been demonstrated to have barrier-strengthening effects on intestinal epithelial cells (2). Moreover, quercetin has a positive effect on intestinal cells during inflammatory challenges (3). In our study, we analyzed the effects of quercetin on porcine VE and FAE regarding the paracellular barrier properties and if the effects differ between both epithelial types.

Methods: For analyzing the effect of quercetin, samples of distal jejunum with and without Peyer's patches were taken from adult pigs and mounted into conventional Ussing chambers. Different concentrations of quercetin, solved in DMSO, were added to the mucosal side (0, 2, 20, 200 and 400 µM), the DMSO concentration for all conditions was 0.1%. During 4 h of incubation under voltage clamp conditions, the transepithelial electrical resistance (TEER) was recorded. In a second approach 0, 200 and 400 µM quercetin were added, and unidirectional tracer flux measurements were performed with [^{3}H]-mannitol.

Results: Whereas lower concentrations of quercetin (2 and 20 µM) had no significant effect on both tissues, the incubation with 200 µM and 400 µM led to a significantly higher TEER in VE (n=15, respectively). For Peyer's patches FAE, no significant change of TEER was observed. Flux measurement did not reveal significant changes, either for both tissues (n=8).

Conclusion: In our study quercetin had an effect on the intestinal barrier of porcine jejunum. Whether the higher TEER values are a consequence of tight junction protein modification, protein regulation as shown in our previous study (2), or if they are caused by the anti-inflammatory effect of quercetin as also shown recently (3), needs to be further elucidated. Different effects in jejunal epithelium and FAE could be caused by the different tight junction protein expression in both epithelia, and a different susceptibility of the tissues to the effects of bioactive compounds.

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23.

Effects of body weight gain on selected serum vitamin levels in ponies and horses

Effekte der Körpergewichtszunahme auf ausgewählte Serum Vitamin Level in Ponys und Pferden

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Lipophilic micronutrients like α-tocopherol and retinol represent an emerging field of research in the context of obesity (1). The concentrations of serum α-tocopherol and serum retinol have not been studied yet in the context of equine obesity. Therefore, we wanted to investigate the course of serum α-tocopherol and retinol under increasing body weight (BW), hereby comparing ponies and horses.

Methods: 10 Shetland ponies (age 6 ± 3 years, mean BW ± SD: 118 ± 29 kg) and 9 Warmblood horses (age 10 ± 3 years, mean BW ± SD: 602 ± 45 kg) were included in the study. All animals started in a non-obese condition according to a body condition score (BCS) on a scale from 0 to 5 (ponies: 2.3 score points; horses: 2.7 score points). In two subsequent years all animals received 200% of their maintenance requirements for metabolizable energy (60% hay, 40% compound feed). During the feeding period, the animals received 2.5 – 3 times the amount of their α-tocopherol requirements and 1.7 – 2.4 times the amount of their retinol requirements provided by the compound feed. BW and BCS were monitored weekly. Blood samples were taken before (t0), after one (t1) and after two years (t2) of excessive energy intake. Serum α-tocopherol and serum retinol were measured using a high-performance liquid chromatography (HPLC) system. Data were assessed for normality by the Shapiro-Wilk test. BW, α-tocopherol and retinol were analysed by ANOVA with repeated measurements. BCS was analysed using the Mann-Whitney U test and the Friedman ANOVA. Statistical significance was accepted at $P < 0.05$. The project was approved by the Ethics Committee for Animal Rights Protection of the Leipzig District Government (No. TVV 32/15). This study is funded by the German Research Foundation (DFG, VE 225/9-1).

Results: Within two years, mean BW increased by 29.9 ± 18.4% in ponies and 16.2 ± 6.3% in horses. Ponies and horses reached a median BCS of 3.8 after two years of BW gain. In ponies serum α-tocopherol increased significantly from t0 (2.35 ± 0.75 µg/mL) to t1 (3.91 ± 1.49 µg/mL) ($P < 0.01$) and from t1 to t2 (6.82 ± 2.22 µg/mL) ($P < 0.01$). In horses serum α-tocopherol rose from t0 (1.78 ± 0.84 µg/mL) to t1 (3.08 ± 0.82 µg/mL) ($P = 0.03$) and from t1 to t2 (4.85 ± 1.44 µg/mL) ($P < 0.001$). Ponies showed significant higher serum α-tocopherol concentrations at t2 compared to horses. In ponies, serum retinol increased significantly from t0 (0.09 ± 0.03 µg/mL) to t2 (0.11 ± 0.02 µg/mL) ($P = 0.02$) and from t1 (0.09 ± 0.01 µg/mL) to t2 (0.11 ± 0.02 µg/mL) ($P = 0.004$). In horses, serum retinol was constant between t0 and t1 and increased significantly from t1 (0.1 ± 0.02 µg/mL) to t2 (0.13 ± 0.03 µg/mL) ($P < 0.001$). Horses showed significant higher serum retinol concentrations at t0 and t2 compared to ponies.

Conclusion: It is a likely explanation that the increase of serum α-tocopherol and serum retinol was related to the high tocopherol and retinol intake by the diets. It was interesting to note that the ponies showed higher serum α-tocopherol concentrations but horses showed higher retinol concentrations. However, the implication on health remained open and further studies are needed on vitamin metabolism in horses.

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24.

Recovery of milk somatic cell count and performance after an intramammary LPS challenge is dependent on the metabolic status of dairy cows

Entwicklung von Milch-Zellzahlgehalt und Leistungsparametern nach einer intramammären LPS-Challenge hängen vom Stoffwechselstatus der Milchkühe ab

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Infections of the mammary gland in dairy cows are commonly accompanied by reduced milk production, feed intake, and poor milk quality. The recovery of the blood-milk barrier functionality after mastitis is crucial to prevent the unintentional exchange of blood and milk components. The metabolic status of early lactating cows is known to affect immune response to pathogens and imposed immune challenges. We investigated to which extent the metabolic status prior to an intramammary LPS challenge (LPS-CH) impacts immune response, milk production and feed intake, and further affects the recovery hereof.

Methods: For 15 Holstein cows, weekly blood sampling and daily recording of dry matter intake (DMI), milk yield, milk composition, and body weight (to calculate energy balance) started 3 wk a.p.. In wk 4 p.p., cows underwent a LPS-CH (50 µg LPS into 1 quarter) with frequent blood and milk sampling. Plasma was analyzed for glucose, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). In milk, serum albumin (SA), immunoglobulin (Ig) G concentration, somatic cell count (SCC) and lactate dehydrogenase (LDH) activity were determined. DMI and milk yield were recorded for a further 6 d. Milk from the LPS treated quarter was sampled for 8 d after the challenge. Based on glucose concentrations in wk 1-4 p.p. prior to LPS-CH, cows were retrospectively grouped into a high (HG, n=7) and low glucose group (LG, n=8). Data were evaluated using mixed models with time, group, time×group interaction as fixed effects and cow as repeated subject.

Results: Glucose was lower (3.17±0.06 vs. 3.68±0.12 mmol/L) and BHB higher (0.99±0.16 vs. 0.71±0.08 mmol/L) in LG compared to HG before LPS-CH ($P<0.05$), while DMI (18.6±1.0 vs. 19.0±0.7 kg/d), energy balance (-35.1±8.1 vs. -26.3±8.2 MJ NEL/d) and SCC (2.1±1.5 vs. 3.0±2.9 [$\log_{10}$/mL]) did not differ. During LPS-CH, SCC (HG: 4.7±0.1 to 7.0±0.3, LG: 4.7±0.1 to 7.2±0.2 at 8 h after the LPS application [$\log_{10}$/mL]) and LDH (HG: 57.9±7.4 to 2,427.4±537.6, LG: 62.8±9.0 to 1,774.3±322.7 U/L at 8 h after LPS application) increased similarly in HG and LG ($P>0.05$), body temperature increased less in HG (40.5±0.4 vs. 41.5±0.2°C after 5 h), BHB (0.72±0.12 vs. 0.64±0.06 mmol/L) and NEFA (0.24±0.06 vs. 0.14±0.04 mmol/L) were higher in LG compared to HG ($P<0.05$). DMI declined in both groups at LPS-CH, but recovered earlier to pre-challenge values in HG (day 1 vs. day 2; $P<0.05$). Milk yield recovered within 2 d after the LPS-CH with no differences in morning milkings, whereas evening milk yield increased faster in HG (day 1 vs. day 2 relative to the LPS challenge day; $P<0.05$). During 8 d after LPS-CH, SCC (5.9±0.1 vs. 6.0±0.1 [$\log_{10}$/mL]), LDH (401±95 vs. 571±98 U/L), IgG (0.39±0.06 vs. 0.47±0.08 mg/mL; $P<0.05$) and SA (0.21±0.06 vs. 0.33±0.07 mg/mL; $P=0.07$) in milk were lower in HG compared to LG.

Conclusion: In conclusion, the metabolic status of cows affects metabolic responses during a LPS-CH as well as the recovery of udder health and performance thereafter.

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25.

Metabolic status and performance at the onset of lactation in dairy cows are associated with circulating serotonin

Stoffwechselstatus und Milchleistung zu Laktationsbeginn von Milchkühen und deren Beziehung zur Blut-Serotoninkonzentration

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For avoiding hypocalcemia and other associated peripartal diseases (e.g., ketosis), a robust regulation of calcium homeostasis around parturition is crucial. Recent studies revealed serotonin (5-HT) as a key regulator of calcium homeostasis in dairy cows (1, 2). Moreover, a role for 5-HT as a regulator of lactation has been proposed. The majority of investigations conducted in transition dairy cows are based on manipulated 5-HT concentrations by infusions of a 5-HT precursor. The objective of this study was to investigate if differences in physiological 5-HT serum concentrations (without exogenous manipulation) are associated with alterations in calcium homeostasis, metabolic status and milk production in dairy cows during the first 14 d after parturition. We hypothesized that higher circulating serum 5-HT concentrations are accompanied by a reduced metabolic load at the onset of lactation in dairy cows.

Methods: Twelve Holstein dairy cows were used to study the impact of physiological differences in serum 5-HT concentrations on the metabolic status and milk production at the onset of lactation. Blood and milk samples were collected at the first 6 morning and evening milkings, starting within 4 h of calving. Sampling was continued at the evening milkings on d 5, 8, 10, and 14. Concentrations of 5-HT, free fatty acids (FFA), beta-hydroxybutyrate (BHB), glucose, calcium, IGF-1 and growth hormone (GH) were measured in blood. Milk was analyzed for fat, protein, lactose and 5-HT concentrations. For comparison of individual serum 5-HT profiles defined by the scheduled sampling events covering the experimental period during the first 14 days of lactation, the area under the curve (AUC) of serum 5-HT concentrations was calculated for each cow using the trapezoidal rule. Cows were retrospectively divided into 2 groups based on their circulating 5-HT concentrations according to the median of the determined AUC. Six cows each were assigned to the high serum 5-HT at a cut-off value of 46,000 ng/mL × 324 h (HSS; AUC of 5-HT [ng/mL × 324 h]: 57,830 ± 4,810; range from 53,719 to 73,674) and the low serum 5-HT group (LSS; AUC of 5-HT [ng/mL × 324 h]: 25,005 ± 5,930; range from 7,522 to 38,142). Parities were 3.7 ± 0.2 for LSS and 3.5 ± 0.6 for HSS, milk yield of the previous lactation was 8,733 ± 392 in LSS and 8,803 ± 1,022 kg in HSS (mean ± SEM). Statistical analysis was performed using SAS (version 9.4). The MIXED procedure was used to evaluate the effects of high and low serum 5-HT concentrations on blood metabolites, milk yield and milk composition with time, group and the time × group interaction as fixed effects and the individual cow as repeated subject. Differences between groups (HSS and LSS) were estimated with the Tukey-corrected t-test. Group differences at individual sampling events were assessed with the TTEST procedure of SAS.

Results: Serum 5-HT concentrations were higher in HSS compared with LSS (7,132±1,823 vs. 2,888±835 ng/mL; $P<0.0001$) during the entire experimental period. The ECM was lower in HSS compared with LSS (12.4±0.9 vs. 14.5±1.4 kg/milking; $P<0.01$). The HSS group produced less colostrum (5.3±0.7 vs. 10.0±1.6 kg; $P<0.05$) and had decreased milk yield ($P<0.05$), specifically during the first 6 milkings. Glucose, FFA and BHB concentrations did not differ between groups. IGF-1 was elevated in HSS compared with LSS throughout the experiment (73.4±13.8 vs. 45.6±6.4 ng/mL; $P<0.001$), whereas GH was concomitantly lower in HSS (4.0±1.4 vs. 9.0±2.7 µg/L; $P<0.001$). Total circulating calcium concentrations in serum tended to be higher in HSS than in LSS ($P=0.12$).

Conclusions: In conclusion, cows with high serum 5-HT concentrations experienced a reduced metabolic load at the onset of lactation, concomitantly lower milk yield, and a reduced energy output via milk. In addition, higher circulating 5-HT concentrations likely improved calcium homeostasis.

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26.

Impact of breed, sex and body mass on feed intake and energy expenditure for growth of young cattle – a meta-analysis of German and Austrian experiments

Einfluss von Rasse, Geschlecht und Lebendmasse auf Futteraufnahme und Energieaufwand für das Wachstum bei Jungrindern – eine Meta-Analyse deutscher und österreichischer Versuche

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In the last decades the milk yield of both Holstein and Simmental has significantly been enhanced by intensive breeding, i.e. selection of animals in terms of dairy type. As a consequence growth and body composition changed, as well. Additionally, frame and body size were increased due to the positive genetic correlation between milk yield and body size. The objective of the present work is to revise the feeding standards for growing and fattening cattle using data of feeding experiments with current types of animals. **Methods:** Data of 42 experiments from nine research institutes in Germany and Austria were pooled and statistically analysed by meta-analysis considering the effect of institute and the experiment within institute. The data provided information on age (98 ± 31, 42–170 days) and live weight (136 ± 35, 80–219 kg) as well as the respective feed intake (DM),nutrient intake (protein, fibre) and energy intake (ME). The data (N = 711) were relatively well balanced regarding breed (Simmental [SI], Holstein [HO]) and sex (male [M], female [F]). The diet consisted of milk replacer (or milk), hay, corn silage and varying amounts of concentrates. The nutrient content of the feeds was on average (in DM): corn silage – 7.7% XP, 41.7% NDF, 10.9 MJ ME; hay – 13.5% XP, 55.1% NDF; 9.4 MJ ME; concentrates – 21.6% XP, 20.7% NDF; 12.4 MJ ME). The mean feed intake was 3.3 ± 1.3 kg DM, the proportion of concentrate 59 ± 18% of DM and mean energy concentration 12.2 ± 1.2 MJ ME/kg DM. A general linear model was used to analyse the fixed effects of breed (SI, HO), sex (M, F) and live weight (90, 110, 130, 150, 170, 190, 210 kg). Additionally, live weight gain (LWG), concentrate intake as well as energy concentrations of forage were used as regression variates. Energy expenditure for growth (ME_g) was calculated as total ME intake minus ME for maintenance (0.53 MJ ME/kg $LW^{0.75}$, GfE 1995). **Results:** Mean feed intake was 3.80 ± 0.02 kg DM/d and mean energy expenditure for growth was 18.6 ± 0.2 MJ ME_g/d (Grand LSMean ± SE of Mean).Regarding both feed intake and energy expenditure for growth, live weight showed the most significant impact ($P < 0.001$). No significant effect of breed on intake and energy expenditure for growth was found. Significant interactions existed for live weight × breed and live weight × sex. The statistical analysis yielded the following results for the effects of breed, sex and body mass:
Mean feed intake for SI = 3.77 ± 0.04 and for HO = 3.83 ± 0.04 kg DM (LSMean ± SEMean)
Mean feed intake for male = 3.75 ± 0.02 and for female = 3.86 ± 0.04 kg DM (LSMean ± SEMean)
Mean feed intake for 90, 110, 130, 150, 170, 190, 210 kg LW = 2.05, 2.61, 3.42, 4.06, 4.48, 4.93, 5.08 kg DM
Mean ME_g for SI = 18.9 ± 0.34 and for HO = 18.3 ± 0.38 MJ ME/kg LWG (LSMean ± SEMean)
Mean ME_g for male = 18.0 ± 0.23 and for female = 19.1 ± 0.40 MJ ME/kg LWG (LSMean ± SEMean)
Mean ME_g for 90, 110, 130, 150, 170, 190, 210 kg LW = 7.8, 12.7, 17.6, 20.8, 22.1, 24.7, 24.6 MJ ME/kg LWG
From the LSMeans for the breed, sex and live weight subclasses minus the Grand LSMean the respective effects can be calculated as well as applied to predict the feed intake or energy expenditure for growth for specific combinations.
Example 1: Feed intake for SI (female, 210 kg LW) = 3.80 – 0.03 + 0.05 + 1.27 = **5.09 kg DMI**
Example 2: Energy for growth for HO (male, 130 kg LW) = 18.6 – 0.3 – 0.6 – 1.0 = **16.7 MJ ME_g**
The residual standard deviation (RSD) and the coefficient of determination (R^2) of the statistical model was 0.24 kg DM and 96.2% for DMI as well as 2.3 MJ ME_g and 87.8% for ME_g. **Conclusions:** The presented models for predicting feed intake and energy expenditure for growth according to breed, sex and live weight show high statistical accuracy (R^2 = 88 – 97%) and can be taken as a guidance for feeding cattle within their growing period from 80 to 220 kg liveweight. A more detailed information about this research can be found in Gruber et al. (2018).

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27.

Effect of dietary L-carnitine in dairy cows on performance and high-resolution measurement of clinical parameters after calving

Einfluss von diätetischem L-Carnitin bei Milchkühen auf Leistung und klinische Parameter nach der Kalbung

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Dairy cows are metabolically challenged during transition period by the drastic increase of energy demanded for milk production on the one hand and the restricted feed intake capacity on the other hand (1). Furthermore, the parturition represents an individual inflammatory challenge (2). The degree of energy deficit and the length of recovery time after calving depend among other things on efficiency of cellular respiration in mitochondria, which in turn starts with import of acyl residue by means of the carnitine-acyltransferase system. The present experiment was designed to clarify the hypothesis that L-carnitine supplemented dairy cows are less affected by the stimulus of parturition as a result of increased efficiency of cellular respiration recognizable by higher stability of clinical parameters and reaching maximum performance earlier.

Methods: For the feeding experiment 60 pluriparous German Holstein cows were divided into a control (CON) and carnitine group (CAR) in consideration of similar numbers of lactation, Body Condition Score (BCS), live weight and fat-corrected milk yield of previous lactation. Experimental feeding started six weeks *ante partum* (ap) with a ration consisting of 80% roughage (70% maize and 30% grass silage) and 20% concentrate on dry matter basis until day one *post partum* (pp). Thereafter, the proportion of concentrate feed was increased first up to 30% and then up to 50% within two weeks pp to reach the final proportion of the lactation feed. The carnitine supplementation (25g/day of rumen-protected L-carnitine, Carneon 20 Rumin-Pro, Kaesler Nutrition GmbH) was applied via concentrate from automatic feeding stations while the remaining ration was fed as a partial mixed ration. To compensate the fat component in the carnitine product, the supplementary concentrate of CON contained an equivalent quantity and quality of fat (Berga-FatF-100 HP 98, Berg+ Schmidt GmbH & Co. KG). Water was offered for *ad libitum* intake during the whole experiment. The data acquisition proceeded till 15 weeks after calving. Before calving, the body weight was recorded once a week, after calving the body weight and the milk yield were determined twice a day and two times per week the milk composition was analyzed. The BCS (3) was recorded once a week over the whole period. After calving the cows were clinically examined at: 0.5, 1, 2, 3, 4, 6, 9, 12, 24, 48, 72 hours (h) pp to determine respiratory rate, heart rate and number of primary rumen contractions. Statistical analyses were carried out by MIXED-Model procedure of SAS 6.1 with time (as repeated measure), group and interaction between time and group as fixed factors.

Results: Carnitine and time showed a significant interaction for milk yield, milk fat and milk protein ($p<0.05$). The overall mean of milk yield in CAR was two kilograms higher as compared to CON during the first six weeks of lactation. For live weight, milk lactose, milk urea, logarithm of Somatic Cell Count a significant time-dependent variation was shown ($p<0.05$), but none of them were affected by carnitine supplementation. The high-resolution measurement of clinical parameters after calving revealed significant differences over time ($p<0.05$) but no carnitine effects. After calving the respiratory rate increased from 43 breath/min (0.5h pp) to 51 breath/min (1h pp) and 50 breath/min (2h pp) returning to initial level after 3 h pp. The number of primary rumen contractions was noticeably changed after parturition ($p<0.001$). Within 3h pp the mean of rumen contractions was 0.6 numbers/2min while it increased up to 2.3 numbers/2min after 72h pp.

Conclusion: The increase in milk yield in early lactation might hint at improved energy utilization in the carnitine supplemented group as compared with the control group. Further evaluations will focus on the energy utilization in more detail to substantiate this hypothesis.

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28.

Effect of dietary L-Carnitine in dairy cows on haematological profiles with special emphasis on parturition

Einfluss von diätetischem L-Carnitin auf die Hämatologie von Milchkühen um die Kalbung

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The transition period is immunological as well as metabolically challenging for dairy cows, in particular parturition itself. During the first 72 hours *post partum* (*p.p.*) various physiological and especially hematological changes occur. A limitation of this approach is the efficiency of cellular respiration in mitochondria. Therefore, acyl residues are transported from the cytosol to the mitochondrial matrix via carnitine transporters. Consequently, a carnitine supplementation leads to better energy supply and therefore the metabolic stress *p.p.* can be reduced. The present study focused on a detailed characterization of red and white blood cell counts and its related parameters in the context of parturition and a potential effect of dietary L-carnitine supplementation.

Methods: For the feeding experiment 60 pluriparous German Holstein cows were divided into a control (CON) and a carnitine group (CAR) in consideration of similar Body Condition Score (BCS), live weight, fat-corrected milk yield of previous lactation and numbers of lactation. Experimental feeding started six weeks *ante partum* (*a.p.*) with a ration consisting of 80% roughage (70% maize and 30% grass silage) and 20% concentrate on dry matter basis until day one *p.p*. Then, the proportion of concentrate was increased first up to 30% and later up to 50% within two weeks *p.p.* to reach the final proportion of the lactation feed. The carnitine supplementation (25g/day of rumen-protected L-carnitine, Carneon 20 Rumin-Pro, Kaesler Nutrition GmbH) was applied via concentrate from automatic feeding stations while the remaining ration was fed as a partial mixed ration. To compensate the fat component in the carnitine product, the supplementary concentrate of the CON group was added with an equivalent quantity and quality of fat (BergaFatF-100 HP 98, Berg+Schmidt GmbH & Co. KG). Water was offered for *ad libitum* intake during the whole experiment. EDTA blood samples were taken on d -42, -14, -3, -1, 1, 2, 3, 7, 14, 21, 28, 42, 56, 100 and 110 relative to calving. Furthermore, frequent samples were taken within the first 12 hours *p.p.* (0.5, 1, 2, 3, 4, 6, 9 and 12h). Red and white blood cell counts and related parameters were determined using an automatic cell analyser (Celltac MEK 6500 α, Nihon Kohden). Statistical analyses were performed using the MIXED procedure of the Software package SAS (9.4) with time (as repeated measure), group and their interaction as main factors and -42 days as covariable.

Results: Carnitine and time showed a significant interaction for platelet counts (p_{group} = 0.376, p_{time}<0.001, $p_{group*time}$=0.027). Within the first 48h *p.p.* carnitine-supplemented cows showed increased platelet numbers compared to control, whereas at the other sampling times there were no differences between both groups. All other haematological parameters showed a significant time-dependent variation (p_{time}<0.001) but none of them were affected by carnitine alone or showed an interaction. Total leukocyte counts (p_{group}= 0.264, p_{time}<0.001, $p_{group*time}$=0.735) showed a constant increase from one week before calving (8.3 G/l), peaking at 4h *p.p.* (14.8 G/l) and returning to initial -42d levels after 48h *p.p.* (7.1 G/l). This temporal development mainly reflected changes in granulocytes. Lymphocytes only showed minor shifts over time.

Summary: The present study documented distinct changes in red and white haemogram of pluriparous dairy cows due to calving in a time frame 7 days prior to until 3 days post calving. Dietary carnitine supplementation significantly increased number of platelets in this period but not in the subsequent lactation period.

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29.

The influence of short chain fatty acids and ß-hydroxybutyrate on the gene expression of gluconeogenetic enzymes in a bovine liver cell line

Einfluss kurzkettiger Fettsäuren und ß-Hydroxybutyrat auf die Genexpression gluconeogenetischer Enzyme in einer bovinen Leberzelllinie

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In dairy cows the energy metabolism is an important factor in different metabolic diseases. The short chain fatty acids (SCFA) acetate, propionate and butyrate are produced in the rumen of cattle and serve as important energy source. The ketone body ß-hydroxybutyrate (BHB) is produced in the rumen wall and in the liver providing energy, particularly when there is a lack of energy. In ruminants gluconeogenesis is essential, because feed carbohydrates are fermented almost completely in the forestomach. The key enzymes of gluconeogenesis in the liver are pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase 1 and 2 (PEPCK1+2) and glucose-6-phosphatase (G6P). The main substrate for gluconeogenesis is propionate. Therefore, propionyl-CoA-carboxylase (PCCB) is the key enzyme for gluconeogenesis from propionate. In our investigations we address the question whether SCFA and BHB affect the gene expression of key enzymes of gluconeogenesis in the bovine liver cell line BFH12 (1). In earlier studies we could show that BFH12 has typical characteristics of liver cells.

Methods: In a first step we optimized growth conditions of BFH12. Therefore we tested different glucose concentrations (2.2 – 5.5 mmol/l), developed an observation protocol and performed a growth curve over 11 days. We also investigated the growth of BFH12 without glucose and without insulin. Afterwards, we run cytotoxicity studies (MTT test) to determine, if there is any adverse effect of acetate, propionate, butyrate or BHB on BFH12 at certain concentrations. In a third step, we evaluated primer sets for the genes of interest and five housekeeping genes (ß-Actin, GAPDH, HPRT, RPL13 und SDHA) with conventional polymerase chain reaction (PCR) and assessed different basic conditions for quantitative PCR (qPCR), including primer efficiency. By the harvesting of samples for qPCR studies there was no added insulin in the media of BFH12 during incubation time. QPCR was performed using the qPCRBIO SyGreen Mix Separate-ROX kit from PCRBiosystems. Statistical analyses were performed with comperative quantitation data from Rotor-Gene Q Series software using REST© (2).

Results: The population doubling time for cells cultured in physiological glucose of 3.3 mmol/l was 24.7 h. Furthermore, no cytotoxic effects on BFH12 were observed for 1000 µmol/l acetate, 250 µmol/l propionate, 20 µmol/l butyrate and 1500 µmol/l ß-hydroxybutyrate. These findings indicate that physiological concentrations of glucose, SCFA and BHB have no negative impact on the viability and proliferation of BFH12. The genes for pyruvate carboxylase, phosphoenolpyruvate carboxykinase 2, propionyl-CoA-carboxylase and the housekeeping genes tested are expressed in BFH12. GAPDH and SDHA were identified as suitable reference genes. Under the chosen experimental conditions, SCFA had an effect on gene expression. Acetate significantly up-regulated all tested enzymes, propionate only up-regulated PC. Butyrate up-regulated PCCB and PC, while BHB had no effect.

Conclusion: In our study we demonstrate that the liver cell line BFH12 grows at physiological concentrations of glucose, SCFA and BHB. We establish qPCR conditions for BFH12. With physiological glucose concentrations and without insulin we observed significant effects of certain SCFA on the key enzymes of gluconeogenesis in this cell line.

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30.

Influence of dietary energy concentration on feed intake and growth performance of fattening Fleckvieh and Braunvieh bulls

Einfluss der Energiekonzentration der Ration auf Futteraufnahme und Leistung in der Bullenmast mit Fleckvieh und Braunvieh

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Previous studies (1, 2) confirmed a high potential for growth in Braunvieh (BV) bulls. Carcass classification as well as energy consumption per kg of body weight gain was, however, considerably better in Fleckvieh (FV) compared to BV bulls. Given that data base on performance and especially on feed intake and nutrient supply of BV bulls is still limiting, the present study was conducted to further evaluate feed intake and growth performance of German FV and BV bulls having a slaughter age of 421 or 467 d comparatively.

Methods: 37 FV (age: 157 d, body weight (BW): 235 kg) and 36 BV (age: 155 d, BW: 223 kg) bulls were allocated by body weight and age at start of the trial to subgroups "energy norm" and energy high". Bulls were fed for ad libitum intake Total Mixed Rations (TMR) based on maize silage and concentrates. Portion of concentrates in TMR of groups energy norm and energy high was set at 20 and 60 % of Dry Matter (DM) to obtain energy concentrations of 11.5 and 12.3 MJ ME (Metabolizable Energy)/kg DM, respectively. Extracted rape seed meal in concentrates was substituted by cereals to obtain comparable CP concentrations of slightly above 14 % of DM in the TMR of the two feeding groups. Individual feed intake was automatically recorded daily while BW was recorded every four weeks. The bulls were slaughtered according to age in two subgroups having a slaughter age of 421 or 467d, respectively. For the statistical analyses a mixed model in SAS was used. The model included the main effects breed, energy concentration of TMR, slaughter age, and interaction of the main effects. Data of 37 FV and 34 BV bulls were used for statistical analysis. Level of significance was set to $p<0.05$ and results are presented as lsmeans ± SE.

Results: DM intake was similar in FV and BV bulls (10.0 kg/d vs. 9.7 kg/d; ±0.17). Daily DM and ME intake was higher ($p<0.05$) in group ME high compared to group ME norm (10.2 and 9.6 kg DM/d; ±0.17; 125 and 110 MJ ME/d; ±2). FV bulls had higher body weight at end of the experiment (729 kg; ±8) and daily gain (1729 g; ±27) than BV bulls (686 kg and 1610 g). There was no difference in end weight and daily gain in animals of group ME high (710 kg and 1676 g) compared to group ME norm (705 and 1663 g). Intake of MJ ME/kg of body weight was higher ($p<0.05$) in BV (74.7 MJ/kg; ±0.9) than in FV (67.5 MJ/kg) bulls, and higher ($p<0.05$) in group ME high compared to group ME norm (75.5 vs. 66.7 MJ ME/kg). Higher ME intake led to an increase of most parameters of carcass fatness (e.g. fat classification, marbling score, intramuscular fat, back fat thickness), whereas those parameters were only slightly increased in BV compared to FV bulls. Weight of kidney fat was, however, higher ($p<0.05$) in BV than in FV bulls in absolute (18.5 vs 16.1kg; ±0.7) or relative (4.9 vs. 3.9 % of carcass weight; ±0.2) terms. Carcass classification (E=1,…, P=5) was better in FV (2.16; ±0.08) than in BV (3.30) bulls. Age at slaughter had no effect on feed intake but daily gain was higher ($p<0.05$) in bulls slaughtered with 421 days of age (1718 g/d; ±27) than in bulls slaughtered with 467 days of age (1621 g/d). Higher age at slaughter led to higher slaughter weight (405 vs. 377 kg; ±5) but also to increased amounts of kidney fat (18.6 vs. 16.4 kg; ±0.7).

Conclusions: Results of the present study confirm the potential for high growth rates in BV bulls, which is, however, lower than in FV bulls. Increased ME intake/kg of body weight in BV compared to FV bulls is in accordance to some measures of body fat content. Increased dietary energy density had no effects on growth or carcass weight but increased body fat deposition. From those data it may be concluded that the chosen high dietary energy concentration of 12.3 MJ ME/kg DM is above a favorable level for both breeds. Economic evaluation of those data is, however still in progress.

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31.

Metabolizable energy content of functional groups in permanent grassland after 50 years field experiment with different levels of N fertilization

Bestimmung der umsetzbaren Energie in funktionalen Gruppen des Dauergrünlandes nach 50 Jahren Feldversuch mit unterschiedlicher mineralischer N-Düngungsintensität

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Permanent grassland provides forages with low production cost. Generally, ruminants in temperate permanent grassland are unable to equilibrate energy and protein of feeds available in the rumen. In consequence, ruminants do not fulfil the requirements for sufficient nutrients and energy to meet their potential genetic in term of milk and meat production. We hypothesize that changes in ME content in each plant functional group (i.e. grasses, legumes, herbs) is an important tool to assist appropriate management strategies in permanent grassland. The aim of this current study was to determine the effect of N application levels on ME value of functional groups in the permanent grassland over the year of 2014 to 2016 after 50 years establishment. After 50 years of field experiment using the same N fertilization levels, the botanical composition is clearly defined, and grasses may benefit the most with N-fertilizer, beyond a certain level.

Methods: The experiment was established at HBLFA Raumberg-Gumpenstein in 1967. The samples for the current study were evaluated from harvesting time of 2014 to 2016. Treatment provision of the fertilizer is as follows: T1, no fertilizer; T2, P_2O_5 (P, phosphorous), K_2O (K, potassium) dynamic; T3, PK dynamic + 80 kg N fertilizer/year; T4, PK dynamic + 120 N; and T5, PK dynamic + 180 N. The experimental design used was a randomized split plot design, with four replications as blocks. Fertilizer application is the main plot and cutting time is the sub plot. A 3-cut system was applied for all treatments since 1993. The ME content was estimated (1), as shown below.

$ME = 5.51 + 0.00828\ ELOS - 0.00511\ CA + 0.02507\ CL - 0.00392\ ADF_{om}$

All the data was subjected to ANOVA using GLM procedure of SAS. Differences were tested using the Tukey-Kramer test and were declared significant at $P<0.05$.

Results: The botanical composition responded to the N-fertilizer addition. Grass is responsive to N-fertilizer, higher level of N-fertilizer consequently increases substantially the proportion grass in permanent grassland ($P<0.01$), legume and herb proportion were reduced significantly due to the higher proportion of grass.

Grasses contribute more to DM yield performance than legumes or herbs, probably due to the dominant proportion in each plot in T3-T5 along the 50 years with each plot receiving the same N-fertilization level. However, ME content of grass, herb and bulk sample declined by the increasing N-fertilizer level ($P<0.01$), as the cutting frequency was kept similar among treatments. The lowest ME content of grass was in T5. There was no difference on the ME content of legumes. Grass responded to N-fertilizer and showed higher and faster growth rates than legumes and herbs, contributing to higher DM yields.

Conclusions: The application of N fertilizer increased the DM yield of permanent grassland after 50 years within the same N level addition. Grasses was the dominant functional group, and they influence substantially the ME yield. The result of this experiment suggest that keeping the 3-cut harvest regime is not suitable for higher ME content although higher DM yield was achieved.

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32.

Time from receipt of a tanniferous extract required to significantly mitigate methane emission from dairy cows

Benötigte Zeit ab Supplementierung eines tanninhaltigen Extrakts für eine signifikante Senkung der Methanemission bei Milchkühen

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The livestock sector is responsible for approximately 16% of the anthropogenic greenhouse gas emissions, which includes the emissions of methane (CH_4). One promising approach to mitigate CH_4 emission in ruminants represents the supplementation of plant secondary compounds such as tannins. Tannins extracted from the bark of *Acacia mearnsii* have been repeatedly shown to be effective in decreasing CH_4 emissions from cattle (1, 2). However, these measurements have been made after supplementation had been carried out for several weeks or even months. The objective of the present study was, therefore, to investigate the immediate response of dairy cows to an *A. mearnsii* extract.

Methods: The study was carried out with 20 lactating Brown Swiss dairy cows averaging 19 ± 3 kg/d of milk and 649 ± 45 kg of body weight. The cows were kept in a tie stall barn and were moved to the respiration chambers to quantify CH_4 production. Methane measurements were conducted over 4 days. During the first day in the chamber, all of the cows received a mixed ration consisting of 55% maize silage, 38% grass silage, 5% protein supplement and 2% hay and up to 1.5 kg/d of grass pellets. In addition, up to 3 kg/d of a commercial energy concentrate served to meet the individual requirements for maintenance and milk yield. Finally, 150 g of a mineral-vitamin mix were supplied. On the second, third and fourth day in the respiration chamber, the commercial energy concentrate as well as the grass pellets were replaced by a concentrate containing 14.1% of an extract rich in tannins prepared from the bark of *A. mearnsii* (Weibull Black, TANAC S.A., Montenegro, Brazil) as well as soybean meal, maize, wheat, wheat starch, and molasses. This concentrate was added to the same mixed ration as fed on day 1. The tannin extract provided by this concentrate accounted for 3 % of the total diet. The total diet crude protein content exceeded 14% on a dry matter (DM) basis. The data were analysed using the Mixed Model procedure (SYSTAT 13) with experimental run and chamber day as fixed effects and animal as random effect. In order to take into account the impact of the difference in dietary NDF content, a second model was run including NDF as a covariate to separate this factor from that of the tannin extract for CH_4 production.

Results: The tannin supplementation had no effect on feed intake and energy-corrected milk (ECM) yield (18 kg/d on average). The CH_4 production (g/d) decreased ($p < 0.01$) from day 1 to days 2, 3 and 4 (from 384 ± 8 to 351 ± 8, 328 ± 8 and 317 ± 8, respectively). The effect was on average 3% lower when corrected for dietary NDF content. On days 2, 3 and 4 compared to day 1, the CH_4 yield (g/kg of DM intake) decreased by 6% ($p = 0.07$), 13% ($p < 0.05$) and 16% ($p < 0.05$), respectively. Additionally, the CH_4 emission intensity (g/kg ECM) decreased numerically, but high standard errors prevented significance.

Conclusion: The results of the current study show that a short-term dosage of 30 g/kg DM intake of an extract obtained from the bark of *A. mearnsii* instantaneously mitigated CH_4 emission without affecting the ECM yield or DM intake in the short-term. The short-term CH_4 reduction was less pronounced than that previously found with long-term feeding (2). However, as the development over the first 3 days indicates, the effect can be expected to become more distinct over time. The supplementation of an acacia bark extract might therefore be a useful nutritional strategy as its effect is immediate (present study) and persistent (2). Its implementation would be easy to accomplish as the product is already manufactured in large quantities for the leather industry.

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This study was supported by the Swiss Federal Office of Agriculture, Braunvieh Schweiz and Qualitas.

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33.

Effect of feeding fresh whey on feed intake and ruminal methane emission of grazing beef cattle

Einfluss der Verfütterung von frischer Molke auf die Futteraufnahme und die Methanemissionen von weidenden Mastrindern

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Whey is a by-product of milk processing to produce cheese. Although rich in energy and minerals, the utilization of whey in alpine regions in a cost-efficient and environmentally friendly way is difficult (1). One possibility to utilize this by-product is to feed it to beef cattle. The aim of the present study was to investigate the effect of feeding fresh whey to grazing beef cattle on feed intake and ruminal methane (CH_4) emission.

Methods: The study was based on a mono-factorial design and was carried out with 20 crossbreed heifers (Limousin × dairy breed) accustomed to the consumption of warm (40°C) full-fat sweet whey and grazing. Based on the body weight (BW) and average daily gain assessed prior to the start of the experiment, heifers were allocated equally to one of two treatments: heifers in treatment GR (BW: 370 ± 37 kg) grazed on a grass-dominated pasture whereas heifers in treatment GW (BW: 370 ± 31 kg) were additionally supplemented with whey for ad libitum intake once daily. For this purpose, animals were fixed in a mobile feeding rack and fed individually using plastic buckets. After a 4-week adaptation to the treatments, heifers of both treatments were divided equally between two consecutive experimental periods so that each heifer underwent a 5 d data collection period. Grass intake was estimated by the double alkane technique (2). Reticular pH was measured continuously in five heifers of each treatment with a wireless telemetric device (eBolus, eCow Devon, Exeter, UK). Ruminal CH_4 emission was determined using the sulfur hexafluoride (SF_6) tracer technique (3). For this purpose, heifers were equipped with a calibrated permeation tube releasing SF_6 two weeks before the measurements started. During the 5-d collection period, respiration gas samples were collected daily into evacuated canisters fixed on the heifers' back; a collection tube with capillary controlled flow was connected with the canister and mounted on a halter such as to position it close to the nostril. Daily CH_4 emission average over the 5 d was calculated from the SF_6 release rate (1.60 ± 0.18 mg/d) and CH_4/SF_6 ratio of the gas sample. Data were analyzed with the ANOVA procedure of NCSS with treatment and experimental period as fixed effect in the model.

Results: The pasture grass had a crude protein (CP) content of 189 ± 23.8 g/kg dry matter (DM) and a net energy content for meat production (NEV) of 6.3 ± 0.2 MJ / kg DM. The CP content of the whey was lower (127 ± 3.3 g/kg DM) and the NEV content clearly higher (10.0 ± 0.1 MJ/kg DM). The heifers consumed on average 2.26 ± 0.73 kg of whey DM per day but as evidenced by the great standard deviation the consumption strongly varied among heifers. Whey supplementation caused a decrease ($P < 0.001$) in grass DM intake of GW heifers compared to GR heifers (3.8 vs 6.4 kg/d) whereas total DM and organic matter (OM) intake did not ($P > 0.05$) differ. The GW heifers had a lower ($P < 0.001$) daily intake of CP and neutral detergent fiber (NDF) and a higher ($P < 0.05$) intake of NEV than GR heifers. The mean reticular pH tended to be lower ($P = 0.06$) in GW heifers (6.34) compared to GR heifers (6.51). The average daily gain and the BW at the end of the experiment did not differ between treatments. Daily CH_4 emission and CH_4 emission per ingested kg OM and MJ NEV were 37, 36 and 45% lower ($P < 0.01$), respectively, in GW heifers compared to GR heifers. No treatment effect ($P > 0.05$) was observed for CH_4 emission per kg NDF ingested.

Conclusions: Although whey consumption varied highly among heifers, CH_4 emission of these heifers was clearly lower compared to unsupplemented heifers without negative effects on fattening performance. The results of the study suggest that whey may be a promising feedstuff to reduce CH_4 emissions from grazing beef cattle not only in alpine regions.

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34.

Relationship between feed intake, efficiency, methane emission, metabolism, lymphocyte activation and lymphocyte proliferation during transition in high-yielding dairy cows

Beziehung zwischen Futteraufnahme, Effizienz, Methanemission, Stoffwechsel, Lymphozytenaktivierung und Lymphozytenproliferation während der Transitionsphase bei hochleistenden Milchkühen

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Immune response largely differs among cows, especially in early lactation, which may also be the result of metabolic and endocrine changes (1, 2). Individual immune response during the transition period, indicated by lymphocyte proliferation, may be related to feed intake, milk yield, methane (CH_4) emission, metabolism, and lymphocyte activation in dairy cows. This was quantified in the present experiment.

Methods: Holstein cows (n=17) in transition to their second (8), third (3), fourth (5) and fifth (1) lactation were studied 14 ± 6 days before and 11 ± 3 days after calving. Dry cows were fed chaffed wheat straw, grass and maize silage, hay *ad libitum* and mineral-vitamin pellets. Lactating cows were fed *ad libitum* on maize and grass silage, protein and energy concentrate, 1 kg hay and salt. In addition, a concentrate-mineral-vitamin-mix was fed on top depend on milk yield. Cows were clinically healthy and classified as low (L, n=6), medium (M, n=5) and high (H, n=6) immune responders based on the proliferation index (PI; L = 1.6, M = 2.2, H= 3.1, SEM= 0.28) of peripheral blood mononuclear cells (PBMC) after calving. The PI is the ratio of 3-[4,5-dimethyldiazol-2-yl]-2,5 diphenyl tetrazolium bromide-reducing activity of stimulated and non-stimulated PBMC (optical density) after 72 h (1,2). The activation index (AI) is the ratio of oxygen consumed (nmol min^{-1} (10^7 cells)$^{-1}$) by stimulated and non-stimulated PBMC after 24 h (2). Glucose (mmol l^{-1}) and cortisol (ng ml^{-1}) concentrations were assessed in blood. Gaseous exchange of cows was measured individually before and after calving for 2 days in respiration chambers to quantify CH_4 production (g day^{-1}), heat production (kJ (kg $BW^{0.75}$)$^{-1}$ day^{-1}) and resting metabolic rate (kJ (kg $BW^{0.75}$)$^{-1}$ day^{-1}). Repeated measurement analyses of variance with the MIXED procedure (SAS 9.3) with time (before/after calving) and group (L, M, H) as fixed effects and the interaction time × group. Multiple comparisons were performed by the Tukey-Kramer test and differences were considered significant at $P < 0.05$ and as a trend at $P < 0.10$.

Results: After calving, feed intake (kg day^{-1}), resting metabolic rate and heat production increased ($P<0.001$), and CH_4 yield (g kg $intake^{-1}$, $P < 0.01$), plasma glucose and cortisol decreased ($P < 0.05$), but no significant differences in these variables among groups and no interaction between time and group were observed. The decrease in CH_4 yield after calving was most pronounced in L cows. After calving, methane emission (g 100 kg body $weight^{-1}$) increased in all groups ($P < 0.01$), but least in L cows. Efficiency of milk production (kg ECM 100 kg body $weight^{-1}$) and of feed conversion (kg milk kg $intake^{-1}$) did not significantly differ among groups. Methane emission intensity (g kg ECM^{-1}) was lower in L than M and H cows ($P < 0.05$). The AI was not affected by group or time, but there was a trend for an interaction between group and time ($P <0.10$).

Conclusion: Cows kept under similar feeding, housing and management conditions and with similar body condition, feed intake, milk yield, feed efficiency and metabolic rate differed in immune response postpartum and in enteric methane emissions. Low responder cows had 20% lower CH_4 yields and 25% lower CH_4 emission intensities than cows with a medium and high proliferative response in early lactation. Because the level of CH_4 emissions is an indicator of rumen fermentation intensity, results suggest that L cows might exhibit a lower fermentation intensity in early lactation. As a consequence, L cows might gain less energy to sustain the shift from lymphocyte activation to proliferation, which might exacerbate their disease susceptibility, because energy is spent without reaching host defense. The selection of low methane emitting cows for environmental reasons could favor animals with a greater disease susceptibility. This has to be monitored.

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The study was supported by the German Research Foundation (SCHW 1485/3-1).

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35.

Animal and environmental factors influencing the β-hydroxybutyrate (BHB)-level in the blood of early lactating dairy cows in commercial farms

Tier- und Umweltfaktoren, die den β-Hydroxybutyrat Gehalt im Blut von Frühlaktierenden Milchkühen in Praxisbetrieben beeinflussen

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Subclinical ketosis (SCK) is described as a precursor for various health disorders in dairy cattle (1). Therefore, early detection is crucial for the treatment of SCK and prevention of secondary diseases. BHB in blood is currently the standard method for the detection of SCK. Commonly applied thresholds vary between 1.0 and 1.4 mmol/L in blood serum (1). The fat:protein ratio (FPR) in milk is discussed to be a suitable indicator for SCK as well (2). The threshold used for FPR is ≥ 1.5. Days in milk (DIM), parity and milk-kg per day (Mkg) are discussed as influencing factors of the extent of body fat mobilization which is causing SCK in early lactation. The aim of this study was to identify additional factors associated with the BHB-Level in serum.
Methods: The study was conducted from April 2017 to March 2018 in 10 commercial dairy farms in northwestern Germany. On each farm 10 early lactating Holstein dairy cows were selected by calving date closest to the start of the trial. Data were collected within two weeks on each farm. Rumenfill score (RFS), Mkg and rectal temperature were documented daily for each cow. Fecal score and blood samples were sampled twice a week. BHB concentration was analyzed in serum. In the evening and in the morning before the blood sample, milk samples were collected and analyzed by mid-infrared spectrometry. From fat and protein in milk the FPR was calculated. All cows were equipped with sensors (RumiWatch) that recorded chewing and activity. The Temperature-Humidity-Index (THI) was calculated from climate data from the housing stalls. Spearman correlation coefficients were used to analyze the relationship of BHB and DIM, Mkg, parity (LN), FPR, RFS, rectal temperature, fecal score and the mean time spend daily for eating, rumination, standing, lying and walking. Further data exploration was conducted by descriptive analysis with PROC MEANS (SAS 9.4). The effects for the model were chosen by backward selection with PROC GLMSELECT with the selection criterion AICc. With PROC MIXED the multiple linear regression model was calculated.
Results: The factors with the highest correlation to BHB were FPR (0.31), Mkg (0.23), lying (0.13), DIM (0.12) and standing (-0.12). The BHB had a minimum (min) 0.15 mmol/L and a maximum (max) of 5.13 mmol/L with a mean of 0.88 (±0.03 SEM) mmol/L. FPR ranged from 0.85 to 3.28 and had a mean of 1.38 (±0.02 SEM). Mkg varied between 10.4 kg/d and 63.8 kg/d with a mean of 37.6 (±0.45 SEM) kg. The mean of DIM was 19.1 (±0.35 SEM) days with a range from 5 to 37 days. A mean of 3.1 (±0.08 SEM) lactations with a min of 1 and a max of 8 lactations were reached. In average, the cows were standing 768 (±7.99 SEM) min/d, the min and max were 293 and 1,220 min/d. The mean eating time was 402 (±4.91 SEM) min/d, the max was 842 min/d and the min was 124,38 min/d. Factors considered in backward selection were: DIM, Mkg, FPR, THI, RFS, rectal temperature, LN, fecal score, eating, rumination, lying, standing and walking time. As effects in the model FPR, Mkg, LN and eating were chosen. By testing these effects in the multiple linear regression model, it resulted in an AICc = 546 and adj. $R^2 = 0.35$. Eating was not significant (P=0.086) thus the model was run again without it. The AICc improved (540) while the adj. R^2 slightly decreased to 0.34. Mkg (P=0.009), FPR (P<0.0001) and LN (P=0.002) were significant.
Conclusion: The correlation between BHB and FPR in this dataset was low but still highest of all tested variables. The early stage of lactation might have been one reason for the low correlation. Therefore, it may be better applied within an index than as a stand-alone especially in the critical first weeks of lactation. The selected effects, FPR, Mkg and parity, explained 34% of the variability of the BHB in serum.

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The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) via the Federal Office for Agriculture and Food (BLE) under the innovation support program.

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36.

Effect of plane of milk replacer feeding on feed intake and methane production from female Holstein dairy calves

Einfluss der Milchfütterungsintensität auf die Methanproduktion weiblicher Holstein Kälber

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During the milk-feeding period, solid feed intake is lower in calves fed a high compared to a low plane of milk replace. After weaning, however, higher solid feed intake levels are observed in calves fed a higher level of milk replacer (1). The level of solid feed intake is known as the main factor influencing methane production in ruminants (2). Thus the objective of this study was to investigate how the level of milk feeding intensity affects methane emission and methane yield in calves pre- and post-weaning.

Methods: Female newborn Holstein calves (n=28) were allocated to 2 feeding groups either fed with 10% (n=14) or 20% (n=14) of their body weight (BW) with milk replacer (MR) after a 3 days period of colostrum feeding. All calves received colostrum from the same pool with one pool for each day. The 10%-MR group was fed with 10% of the BW with colostrum and the 20%-MR group with 12% colostrum, respectively. Hay was offered ad libitum until week (wk) 14 of age. Starter was offered ad libitum until wk 12 of age with a following 2 wk period of step-down to 2 kg per day and another following 2 wk period of gradual reduction to 0 kg. A total mixed ration (TMR) was fed ad libitum from the age of 10 wks until the end of the trial at wk 22. Milk replacer feeding of the 20%-MR group was gradually reduced to 10% of the BW from wk 8 to 10 of age. Gradual weaning for both groups took place from wk 10 to 12 of age. Feed intake was measured daily and BW weekly. Methane production was determined 2 times before weaning and 2 times after weaning for 48h at the age of wk 5 (37d ± 3), wk 8 (58d ± 3), wk 13 (93d ± 4) and wk 21 (150d ± 3) in respiratory chambers. The statistical analysis was performed using the MIXED procedure of SAS with repeated measures. Dry matter intake (DMI) and daily methane production (L/d) were analyzed separately for the pre-weaning (wk 1-12) and the post-weaning (wk 13-23) periods.

Results: The BW increased in both groups over time ($P \leq 0.05$), but to a higher extent in the 20%-MR than 10%-MR group ($P \leq 0.05$). In the pre-weaning period, solid feed DMI was higher in the 10%-MR than in the 20%-MR group ($P \leq 0.05$), whereas post-weaning the 20%-MR group had higher means ($P \leq 0.05$). Solid feed DMI per kg BW was persistently higher in the 10%-MR than 20%-MR group until the end of the trial. According to the different DMI level, the 10%-MR group showed higher daily methane production in the pre-weaning period ($P \leq 0.05$), whereas the 20%-MR group tended to show higher means post-weaning ($P \leq 0.05$). For both dietary groups daily methane production increased over time ($P \leq 0.05$). When methane production was normalized to BW, values for both groups increased over time ($P \leq 0.05$) and were constantly higher in the 10%-MR compared to the 20%-MR group ($P \leq 0.05$). Methane emission per unit of solid DMI (methane yield) tended to be higher in the 20%-MR than 10-%MR group ($P \leq 0.1$) and decreased over time in both groups ($P<0.01$).

Conclusion: Methane emissions from calves increase with the increase in BW and DMI. A higher plane of MR feeding increases daily methane production after weaning and tend to increase methane yield.

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37.

Influence of a vegetal carbon supplementation on methane emissions of dairy cows in late lactation

Einfluss einer Supplementierung mit pflanzlicher Kohle auf die Methanemission spätlaktierender Milchkühe

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Up to 12% of energy intake in dairy cows might get lost due to methane emission (1). In order to improve the energy balance of dairy cows a reduction of methane emissions might be reasonable. The methane emissions of cattle are highly dependent on the composition of the ration and might be reduced by different dietary supplementations. Vegetal carbon has been proven to reduce methane production *in vitro* (2) at 1.5% of dry matter. The aim of this experiment was to investigate the influence of a vegetal carbon supplementation on methane emissions of dairy cows in late lactation *in vivo*.

Methods: Twenty five pluriparous German Holstein cows (225 ± 28 days in milk; means ± SD) were allocated to a control group (C; n=11) and a group supplemented with vegetal carbon (BC; n=14). The BC-group (n=14) received 250 g/d (1.25% of dry matter intake (DMI)) of vegetal carbon (CharLine, Manufacturer CharLine GmbH, Riedlingsdorf; 26 % XA, 5 % XP) for five continuous weeks. The powdered vegetal carbon was mixed into the concentrate. The forage consisted of 30 % maize silage and 70 % grass silage. Methane emissions of both groups were measured by a GreenFeed system for five continuous weeks. Cows were allowed to enter the GreenFeed five times daily for five minutes with a minimum time interval between visits of three hours. On each visit cows received 272 g of concentrate. The statistical analysis was performed using the MIXED procedure of the SAS software (9.4) for repeated measures with a compound symmetry structure. The factors in the model were group, time and the interaction between them. *P*-values < 0.05 were considered to indicate significant differences. Results are presented as LSMeans ± SEM.

Results: We observed a trend towards a higher DMI in the C-group (19.7 ± 0.3 vs. 19.0 vs 0.3 kg/d; $P = 0.069$). There was a trend of energy corrected milk (ECM) being higher in the C-group (33.1 ± 0.6 vs. 31.7 ± 0.5 kg/d; $P = 0.084$). Absolute daily methane emissions were influenced by time ($P < 0.001$). The highest daily emissions were observed in week three for both groups (487 ± 20 g/d for the C-group; 456 ± 17 g/d for the BC-group) due to a changed XF-intake during the trial. Time influenced methane emissions per kg DMI ($P < 0.001$), with the highest relative emissions being observed in week three (28 ± 1 g/kg for the C-group; 26 ± 1 g/kg for the BC-group). Methane emissions per kg ECM were influenced by time ($P < 0.001$). The highest relative emissions were observed in week three (15 ± 1 g/kg for the C-group; 14 ± 1 g/kg for the BC-group). Vegetal carbon did not affect absolute and relative methane emissions.

Conclusion: The results of the present trial indicate that the vegetal carbon did not elicit an effect on methane emission which contradicts in vitro results (2). This might be associated to the dosing and composition of vegetal carbon. However, higher doses might negatively affect feed intake.

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38.

Effects of *Scrophularia striata* extract on *in vitro* nutrient degradation and CH_4 formation when added to a diet containing a low-quality fibre source

Auswirkungen von Scrophularia striata Extrakt auf die in vitro Nährstoffverdaulichkeit und CH_4-Bildung beim Zusatz zu einer Ration mit einer minderwertigen Faserquelle

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In terms of a sustainable meat and dairy production, by-products of the food production chain are gaining importance as feedstuffs for livestock animals. The bio-refinery that uses alfalfa silage juice as amino acid source generates a fibrous by-product (alfalfa silage cake; AC), which according to our hypothesis can be used as nutritional fibre source for ruminants. However, crude protein (CP) and energy contents are low and additional concentrate is needed to improve dietary nutrients. *Scrophularia striata* is a herbaceous flowering plant, which has long been used as a medicinal herb in some Asian countries. The extract of *S. striata* is rich in phenolic and flavonoid compounds (1) and has previously been shown to have antimicrobial properties, resulting in decreased CH_4 formation and decreased CP degradation and ammonia concentrations when added to a diet containing high-quality hay in *in vitro* rumen simulation technique (Rusitec) fermenters (2). Now, we wanted to investigate the effects of *S. striata* extract (SE) when added to a diet with a low-quality fibre source like AC.

Methods: Three forages, the bio-refinery by-product alfalfa silage cake (AC), the original alfalfa silage (OA) and a fiber-rich hay as control (Hay) were tested each as part of a total mixed ration either with or without supplementation of freeze-dried SE (60 mg/g of DM) in a Rusitec-system. The forages differed in CP (g/kg dry matter (DM): AC = 120, OA = 190 and Hay = 70). While NDF content (710 g/kg DM) of AC and Hay was similar, ADL content was considerably higher in AC than in Hay (87 g/kg DM vs. 48 g/kg DM). Concentrates were formulated to balance for similar contents of CP (169 g/kg DM) and non-fibrous carbohydrates (250 g/kg DM) in the unsupplemented diets. The Rusitec experiment consisted of 3 runs, each one lasting 10 days with the last 5 days serving as sampling period. Samples were analyzed for concentrations of ammonia and short chain fatty acids (SCFA) as well as for pH-value and redox potential. In addition gas production and nutrient degradability of the incubated diets were measured after 24 and 48 hours of incubation, respectively. Statistical analysis was performed with Proc Mixed of SAS (9.4) using a 3×2 factorial design (three diets and two treatments: no SE or supplementation of 60 mg/g DM SE).

Results: Fermenter pH was in tendency affected by diet ($P=0.071$), while redox potential increased significantly when SE was included ($P<0.001$). While NDF degradability of all diets was similar (29.4%), degradability of CP was much lower with the AC diet compared to the others (diet effect $P<0.001$). Both, degradability of CP and NDF were strongly reduced due to SE supplementation in the AC (-12% and -31%) and Hay (-13% and -29%) diets (effect of SE on CP and NDF degradability, respectively: $P=0.002$ and $P=0.018$). Consequently ammonia concentration was lower when SE was supplemented ($P<0.001$), whereas concentrations of short chain fatty acids remained unaffected. Unexpectedly, CH_4 formation was not inhibited by SE, instead it differed between diets ($P=0.013$) with a trend for a diet×SE interaction ($P=0.087$). Supplementation of SE tended to inhibit CH_4 formation with the AC (-22%) and Hay (-10%) diets, while CH_4 formation was increased when SE was added to the OA diet (+25%).

Conclusions: NDF degradability was similar between diets, indicating AC's suitability as nutrient fiber source for ruminal microbes. Supplementation of SE decreased *in vitro* ruminal NDF and CP degradability and ammonia concentrations. Effects of SE supplementation on ruminal methane formation depended on the dietary composition, as inhibitory effects were only prominent when SE was supplemented to the diets containing AC and Hay but not when added to a diet rich in alfalfa silage. Further research is necessary to elucidate the interaction of SE with dietary components and their effects on the ruminal microorganisms that are involved in ruminal nutrient degradation.

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39.

Effects of a mixed-nematode infection on vitamin A and E and carotenoids in plasma, liver and egg-yolks in laying hens

Einfluss einer Misch-Nematoden-Infektion auf Vitamine A, E sowie Carotinoid-Gehalte in Plasma, Leber und Eigelb von Legehennen

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Gastrointestinal nematode infections in layer chickens are highly prevalent (1). Vitamin-A has been reported to mitigate the impact of nematode infections in chickens. Carotenoids have been associated with egg-yolk colorization, enhancing humoral immune responses and lower oxidative stress in chickens. In order to assess whether nematode infections affect the major fat-soluble antioxidants in different tissues of high performing laying hens, we compared chickens experimentally exposed to nematode infections with uninfected controls for vitamins A and E and carotenoids levels in different tissues. We hypothesized that nematode-infected chickens will have lower fat-soluble antioxidant reserves due to a diverted allocation toward defense mechanisms at the expense of storage in liver and egg yolks.

Methods: Eighteen laying hens (Lohmann Brown plus) were experimentally infected with *Ascaridia galli* and *Heterakis gallinarum* at an age of 24 weeks. Un-infected control hens (n=17) were also kept in separate pens of the same experimental stable. Hens were fed a commercial layer diet ad libitum. Vitamins A, E and total carotenoid contents in the diet were 0.23, 34.04 and 1.29 ppm, respectively. The hens were necropsied at 2, 4- and 6-weeks post infection (wpi) to determine their infection intensity and collect individual blood, liver and egg-yolk samples. Plasma, liver and egg-yolk samples were analysed for vitamin A (retinol), vitamin E ((i.e. tocopherols (alpha, gamma tocopherol) plus tocotrienols (alpha, gamma)) and total carotenoid contents using HPLC. Individual carotenoid (i.e. lutein, zeaxanthin, canthaxanthin and apo-ester) concentrations were further determined in egg yolks. Co-enyzmeQ10 (Co-eQ10) was also determined in liver and egg-yolk samples. Pen-based laying performance, feed intake, feed:egg mass were assessed at weekly intervals. Egg-yolk pigmentation was measured according to CIE-L*a*b* using a Chroma Meter. Hen individual data were analysed with a 3-way-ANOVA to account for the effects of infection, tissue, wpi and the two- and three-way-interactions plus individual animal effect using Proc Mixed of SAS (V9.4). Pen-based performance data were analysed similarly with omitting the tissue effect and using pen as the experimental unit.

Results: All infected hens harbored both *A. galli* (29±18 worms / hen) and *H. gallinarum* (203±135 worms / hen) at infection intensities that were highly comparable to the levels observed in naturally occurring infections (1). Infection impaired (P=0.018) overall laying performance by 7.7 % (i.e. from 95.1 to 87.7%) mainly due to a lower (P=0.061) feed intake (ca. 5.5%), but not feed:egg mass ratio (P=0.404). Egg yolks of the infected hens had a lower (P=0.033) redness value (a*) than that of uninfected control hens. Infection reduced concentration of retinol in liver (P<0.05) but not in other tissues (P>0.05). Although liver retinol concentrations correlated negatively with *A. galli* counts, ranging from -0.39 to -0.68 from wpi 2 to wpi 6, the correlations were not significantly different from zero (P>0.05), likely due to low numbers of infected hens per wpi (n=6). Concentrations of lutein, zeaxanthin, canthaxanthin, apo-ester and total carotenoids in egg yolks remained unaffected by the infection effects (P>0.05). Except for delta-tocopherol, concentration of all measured variables was lower (P<0.05) in plasma than in liver or egg yolks.

Conclusions: Although infection-induced lower feed intake may partly explain lower retinol reserves in liver, concentrations of carotenoids, vitamin-E derivatives and Co-eQ10 remained unaffected in relevant tissues. This may imply an elevated requirement of vitamin-A but not other fat-soluble antioxidants in the infected animals, likely due to a diverted allocation of vitamin-A toward defense mechanisms at the expense of storage in liver.

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This study was funded by the German Government´s Special Purpose Fund held at Landwirtschaftliche Rentenbank.

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40.

Effects of feeding chopped hay and grass-clover silage on feed intake, apparent total tract digestibility, and performance of organic dairy cows

Effekte von vermahlenem Heu und nachzerkleinerter Grassilage auf die scheinbare Verdaulichkeit der Nährstoffe sowie die Futteraufnahme und Leistung von Milchkühen unter ökologischen Bedingungen

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Current EU legislation for organic production encourage reduction of concentrates in dairy cows' diets. When feeding bulky diets, a high feed intake is essential to meet the cow's nutrient requirements. Although reduced forage particle size (RFPS) is known to stimulate feed intake, this might decrease particle retention time and thus feed digestibility. Recently, a meta-analytical study (1) showed that effects of RFPS were modulated by the type, inclusion level, and preservation method of forages. Since organic rations contain a minimum of starch, effects might be different than reported by studies using grain-rich diets. The current study aimed to investigate the effects of RFPS on dry matter intake (DMI), apparent total tract digestibility (ATTD), and animal performance.

Methods: 20 Holstein cows were balanced by average milk yield (26.7 kg), body weight (703 kg), days in milk (135 d), and number of lactations (3.8) into 2 feeding groups. CALAN gates were used to record individual feeding intake. After a covariate period (7 d), groups were switched to a TMR, either with a conventional particle size (CON) or RFPS (RED) for 34 d, including an adaptation period of 12 d. TMR contained equal proportions (on DM basis) of hay (43%), grass-clover silage (37%), and concentrate (20%), resulting in 6.92 MJ NE_L, 151 g CP, 35 g starch, and 437 g NDF per kg DM. Before preparing the RED ration, hay and silage were chopped at theoretical length of cut of 0.5 cm and hay additionally hammer-milled (2 cm sieve). Cows were offered fresh TMR after milking (06:00 and 17:00), allowing for 10% feed refusals. Particle size was determined using 3 screens (19 mm, 8 mm, 1.18 mm) and a pan on a daily basis as well as DMI and milk yield. ATTD was determined using acid insoluble ash as an external marker. Nine fecal grab samples were taken from each cow's rectum at 8 h intervals starting 5 days before the trial ended. ANOVA was done with proc mixed (SAS 9.4), considering an effect of the feeding group, day and covariates for the corresponding parameter whereas cow nested within the group, was considered as random effect.

Results: Particles retained on the screens and the pan accounted for 73.5, 9.6, 11.9, and 5% in CON, and for 23.0, 26.4, 36.8, and 13.8 % in RED on a DM basis, respectively. Cows reached a reasonable DMI level in CON (21 kg/d), but the level increased further (+1.8 kg/d) when feeding the RED diet ($P < 0.05$). The ATTD of nutrients was improved significantly when feeding chopped forages, but fecal DM remained constant. The digestibility of OM and NDF increased 4.8 and 3.3 % points, starting at 71.6 and 70.4 % in the CON group, respectively. As a result, RED cows' energy-corrected milk (ECM) production (27.0 vs. 29.3 kg/d) and daily gain (0.34 vs. 0.81 kg/d) increased. Results revealed also that chopped forages improved ($P < 0.05$) estimated energy balance (110 vs. 115%) even without considering enhanced ATTD. Feed conversion efficiency (kg ECM/kg DMI) and N use efficiency were not affected by the treatments.

Conclusions: The improvement of DMI and digestibility in the RED cows suggest a better forage utilization by RFPS feeding, likely due to an increased particle surface and probably due to prolonged entrapment of particles in the ruminal mat. The data indicate that chopping forages is beneficial in organic dairy production, when diets contain limited concentrate levels. Since this study tested only 1 RFPS more research is required to optimize the RFPS in organic cattle diets and also technically to produce forages with RFPS in larger production scale.

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The study was funded by the BMNT, Land Salzburg, Raiffeisen Salzburg, and the "Ja! Natürlich" brand of the REWE group.

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41.

Effects of concentrate proportion of the ration on rumen fermentation, rumination activity and the risk of a subacute ruminal acidosis in early lactating dairy cows

Einfluss des Kraftfutteranteils der Ration auf die Pansenfermentation, die Wiederkauaktivität sowie das Risiko einer subakuten Pansenazidose bei Milchkühen während der Frühlaktation

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In early lactation, the imbalance between insufficient increase of energy intake and high energy requirement induces a negative energy balance (**NEB**). To compensate the NEB, the concentrate proportion of the ration is increased, which can enhance the risk for subacute ruminal acidosis (**SARA**). The ruminal pH is used for detecting SARA, but often considered critically as individuality of cows is not included. The aim of the study was to examine whether pH should be considered in context of other parameters, such as behavior and volatile fatty acids (**VFA**) to describe rumen conditions more adequately. Therefore, ruminal pH and ruminating activity were measured simultaneously and VFA were determined under different concentrate proportions (**C**).

Methods: Rumen-fistulated dairy cows were divided into a low and a high concentrate group, with rations containing 35% (n=7, $\mathbf{C_{35}}$) or 60% concentrate feed (n=6, $\mathbf{C_{60}}$), after calving. For the latter, C was gradually increased from 35% to 60% during the first three weeks post partum (**p.p**.). Before parturition, all cows received the same ration consisting of 80% silage (56% maize silage, 24% grass silage) and 20% concentrate on a dry matter (**DM**) basis. DM intake (**DMI**) and milk yield were recorded and milk samples were collected twice a week. A continuous ruminal pH measuring device recorded the pH values (Lethbridge Research Centre Ruminal pH Measurement System, Dascor, Escondido, CA, USA). Ruminating activity was determined by a noseband pressure sensor (RumiWatch, Itin + Hoch GmbH, Liestal, Switzerland). The pH values as well as rumination of each cow were recorded every minute and measured during several consecutive 24-h periods each week (2 ± 1.16; mean ± SD). To identify SARA, thresholds according to (1) were applied. A pH value <5.8 for 314 min/d, as well as a daily mean pH of <6.16 were considered to diagnose SARA. VFA concentrations were measured in rumen fluid samples collected ventrally on 8 time points. Data were analyzed using the MIXED procedure of SAS Enterprise Guide 6.1 with fixed effects of concentrate and time and the interaction between them.

Results: The average p.p. DMI amounted to 19.2 kg/d ± 3.6 and 21.1 kg/d ± 2.2 (mean ± SD) in groups C_{35} and $C_{60,}$ respectively. Five out of 7 cows of group C_{35}, and 6 of 6 cows of group C_{60} were diagnosed to suffer from SARA, whereby the incidences were not significantly different between groups (314 min at pH <5.8 : $P = 0.565$, daily mean pH <6.16: $P = 0.701$). Ruminate chews as well as ruminated boluses were influenced by C, as increased concentrate had a negative effect over time ($P_{\text{ruminate chews}} = 0.025$, $P_{\text{ruminated boluses}} = 0.023$). A higher C tended to decrease the ruminating time over time ($P = 0.094$). The enhanced concentrate amount tended to increase propionate and decrease acetate over time ($P_{\text{propionate}} = 0.053$, $P_{\text{acetate}} = 0.053$). These results were accompanied by a decreased milk fat content due to an increased C ($P = 0.010$). C_{60} tended to have lower ECM values ($P = 0.093$).

Conclusion: In the present investigation, both groups achieved the defined thresholds for SARA. Although C_{60} exhibits more critical values, the differences between groups were not significant. Contrary to the expectations, relatively low C also led to critical rumen pH values that could indicate SARA under the given conditions. The diet with a lower amount of concentrate led to an increase in ruminating activity, which in turn is known to stimulate the saliva production and therefore entails buffering properties. These effects did obviously not seem to compensate the decreasing ruminal ventral pH values. C_{35} exhibited a higher acetate concentration and therefore a higher milk fat content. This group also showed higher ECM values, despite lower DMI. This superior performance might indicate an improved rumen fermentation and is not reflected in the critical pH values. Further research is necessary to clarify the relation between rumen pH and other parameters.

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42.

Effect of varying trypsin inhibitor activity in differently processed soybean cakes on praecaecal amino acid digestibility in broilers

Einfluss einer variierenden Trypsininhibitoraktivität unterschiedlich aufbereiteter Sojakuchen auf die praecaecale Aminosäurenverdaulichkeit bei Broilern

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Several crop plants contain a number of anti-nutritional factors (ANF). Especially raw soybeans can express a high trypsin inhibitor activity (TIA), which may impair animal health and productivity. Therefore, soybean products have to be treated by heat and pressure to reduce this anti-nutritive potential. Preliminary results indicate that even a low TIA has measurable impacts on animal performance and productivity. The present study took the next step by investigating the effects of varying TIA on praecaecal digestibility of amino acids.
Methods: Raw material for this experiment consisted of two homogenous batches of soybeans Sultana (native TIA: 37.3 mg/g) and Merlin (native TIA: 40.5 mg/g). Four processing techniques (thermal, hydrothermal, pressure, kilning) were used for creating forty-five differently treated soybean cakes. One basal diet was formulated which served as control diet (15.8% crude protein of total feed). The processed soybean variants were then merged into the basal diet and experimental diets contained either 15% or 30% processed soybean cake with concurrent reduction of maize starch. In this way, ninety experimental diets were developed. All diets contained 0.5% of titanium dioxide. The TIA in the feed varied from 0.4 mg/g to 8.5 mg/g in total feed. A total number of 5,490 1-day-old male broiler chickens (Ross 308) were used in four consecutive trials and were allocated to groups of 10 birds to 140 pens (1,6 m^2). For the last experimental run, only 129 pens were used instead of 140. From day 1 to day 14 the birds were fed with a commercial starter diet (crude protein 215 g/kg, 12.5 MJ ME/kg). From day 15 onwards, the experimental diets were provided ad libitum. At the end of the experiment (day 21) the birds were weighed individually and euthanized by asphyxiation with carbon dioxide. Digesta sampling occurred according to (1). Amino acid contents of feed and digesta as well as titanium dioxide were analysed and digestibility coefficients were calculated due to standardized methods (2). Regression models were designed to characterize the effect of TIA to the praecaecal digestibility of single amino acids.
Results: TIA depressed the apparent praecaecal digestibility of every single amino acid significantly ($p < 0.001$). Cysteine (Cys) and Methionine (Met) expressed the strongest reaction to TIA, with Cys showing the lowest digestibility coefficients measured (19.82% digestibility at TIA of 7.9 mg/g). Lysine (Lys), in contrast, was the least affected amino acid. Consequently, the ratio of Met+Cys to Lys showed the same significant ($p < 0.001$) depression with rising TIA trend. As expected, performance data fit to these findings. Body weight ($p < 0,001$), total weight gain ($p < 0,001$) and daily weight gain ($p < 0,001$) declined in a linear manner with increasing TIA in feed.
Conclusion: The present data imply that TIA has major effects on essential and non-essential amino acid availability and thus on growth performance. Hence, considering the amount of anti-nutritional factors is important when planning the supplementation of crystalline amino acids. This underlies that even the lowest TIA has measurable effects, so reducing TIA in feed to a minimum is essential to avoid a decline in performance.

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The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support program (ptBLE FKZ: 2814EPS022).

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43.

Effects of type of concentrate and timing of supplementation on feed intake, milk performance, and nitrogen use in cows grazing an alfalfa-rye grass sward

Einfluss der Art des Konzentratfutters und des Zeitpunktes der Supplementierung auf die Futteraufnahme, Milchleistung und Stickstoffverwertung von weidenden Kühen auf einer Alfalfa-Weidelgras-Weide

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Rapid ruminal degradation of protein in alfalfa forage generally leads to poor efficiency of nitrogen (N) utilization in grazing dairy cows. Both, N use and rumen microbial crude protein synthesis (MPS) can be improved by supplementing non-structural carbohydrates to cow diets (1). However, the effects may differ depending on the degradation rate of the carbohydrate source and timing of supplementation (2). The aim of this study was therefore to evaluate the effects of two cereal grains differing in ruminal starch degradation and timing of supplementation on feed intake, diet digestibility, rumen MPS, N use efficiency, and milk performance of lactating cows grazing an alfalfa-rye grass sward.

Methods: A feeding trial was conducted in the Peruvian Andes using 24 lactating Brown Swiss cows (21 multiparous, 3 primiparous) in an incomplete 3x4 Latin square design with three periods of 21 d (14 d adaptation, 8 d sampling). Cows had (mean±standard deviation) 458±48 kg live weight (LW), 141±52 days in milk, and 15±2 kg/d milk yield at the start of the trial. All cows grazed an alfalfa-rye-grass sward for 8 h/d. They were allotted to four groups of six cows each and assigned to one of four dietary treatments. Dietary treatments included supplementation of 3 kg/d of either corn (C) or oat (O) meal plus 0.5 kg/d of corn cobs. Concentrate mixtures were offered (as-fed basis) at 1 kg/d during morning and 2.5 kg/d during evening milking (i.e., CPM, OPM) or at 2.5 kg/d during morning and 1 kg/d during evening milking (i.e., CAM, OAM). Faecal organic matter (OM) excretion was determined using titanium dioxide as external marker and apparent total tract OM digestibility (OMD) was estimated using the equation of Lukas et al. (2005) (3). Total OM intake (OMI) was calculated from the OMD and faecal OM excretion. Urine volume was estimated from urinary creatinine concentration and the urinary purine derivatives excretion determined to estimate rumen MPS. LW was measured at the beginning and at the end of each period. Individual milk yield was recorded daily. Data were subjected to ANOVA using GLM procedure of SAS with contentrate type, timing of supplementation, and their interaction as fixed effects. Pairwise comparisons of least square means were made by PDIFF option, and effects considered significant if $P \leq 0.05$.

Results: Total OMI (kg/day) was similar ($P > 0.05$) for all treatments (CPM=12.7, CAM=12.9, OPM=12.4, OAM=12.4). An effect ($P \leq 0.05$) of cereal source was observed for OMD (g/kg OM) with CPM=800, CAM=784, OPM=769, and OAM=773. Rumen MPS (g N/d) was CPM=144, CAM=125, OPM=132, and OAM=139 and did not differ between concentrate mixtures ($P > 0.05$) or timing of supplementation ($P > 0.05$). However, an interaction effect was observed ($P \leq 0.05$). The N use efficiency (g milk N/g N intake) was CPM=0.37, CAM=0.30, OPM=0.36, and OAM=0.37 without any differences between treatments ($P > 0.05$). Dietary treatments also did not affect animal LW (CPM=462, CAM=464, OPM=467, and OAM=473 kg/cow; $P > 0.05$) and daily milk yield (CPM=15.5, CAM=14.7, OPM=14.1, and OAM=15.1 kg/cow; $P > 0.05$).

Conclusions: Timing of supplementation and type of concentrate feed influence OMI, OMD, and rumen MPS in lactating cows grazing an alfalfa-ryegrass sward. Such effects however, do not necessarily enhance animal performance and N use efficiency.

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44.

Effect of roughage energy and concentrate level on characteristics of feed / energy intake and milk yield by Holstein and Simmental dairy cows

Einfluss von Grobfutterenergie und Kraftfutterniveau auf Merkmale der Futter- und Energieaufnahme sowie der Milchleistung bei Holstein- und Fleckviehkühen

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The aim of this study was to show the effect of the energy level in roughage and the concentrate level on feed / energy intake, milk yield as well as energy balance by Holstein and Simmental dairy cows.

Methods: Two levels of roughage energy, a variant with 6.1 MJ NEL / kg DM and a variant with 6.5 MJ NEL / kg DM were supplied. A reduction of energy to reach the low energy level was achieved by adding straw. The use of concentrate was varied with a low level of 150 g / kg ECM (L) and a high level of 250 g / kg ECM (H). The energy level of the concentrate was 7.0 MJ NEL / kg DM. Daily amount of concentrate was fed according to the desired level of the experimental group, depending on the expected milk yield, lactation status and lactation number (first / later). Four experimental groups were studied in this feed trail (6.1 L, 6.1 H, 6.5 L, 6.5 H). Data were recorded on three experimental farms for each breed for about two years within the project optiKuh. In total, data from 117 Holstein and 221 Simmental dairy cows were available. The allocation of the Holstein cows to the experimental groups was 37 cows in 6.1 L, 54 cows in 6.1 H, 36 cows in 6.5 L and 50 cows in 6.5 H. For the Simmental cows it was 21, 25, 87 and 88, respectively. Observations were weekly averages, which were determined on the basis of daily recordings during the experimental period. The statistical analysis included observations from the 5^{th} to the 350^{th} lactation day. Data was evaluated separately by breed. The program package SAS with the procedure MIXED and the method REML was used for statistical analysis. In the mixed model as fix effects, the herd test week, the experimental group, the lactation number and the lactation day within the lactation number and as random effect, the cow was considered. The lactation day was modeled with the function according to ALI and SCHAEFFER (1). The level of significance was set to $p \leq 0.05$. The energy balance was calculated on the basis of MJ NEL and MJ ME taking into account recent findings of the ME requirement and recovery.

Results: In all investigated characteristics of feed / energy intake and milk yield the experimental group 6.5 H differed significantly from the experimental group 6.1 L ($p \leq 0.05$). The experimental group 6.5 H had higher LSM values than group 6.1 L. The Holstein cows in the experimental group 6.5 H had a feed intake of 22.7 kg DM / day and in the experimental group 6.1 L 20.6 kg DM / day. The Simmental cows 20.6 kg DM / day and 18.4 kg DM / day respectively. The energy supply of the animals at the level of the experimental group 6.1 L, considering the energy balance, was inadequate, especially for heifers. In this experimental group the energy balance for Holstein cows was - 0.5 MJ NEL / day and - 8.3 MJ NEL / day for Simmental. There were no significant differences between the experimental groups 6.1 H and 6.5 L ($p > 0.05$). The experimental groups 6.5 L and 6.5 H did not differ significantly from each other in terms of milk yield, but with regard to the feed / energy intake and energy balance ($p \leq 0.05$). All characteristics had higher LSM values in the experimental group 6.5 H compared to 6.5 L. Within single lactation numbers the respective findings for significant differences between the experimental groups differed from the overall mean.

Conclusions: It can be stated that both, high energy level in roughage and a high concentrate level can have a positive effect on feed / energy intake, milk yield and the energy balance of dairy cows. If further evaluations like health characteristics and the economy are taken into account, it could be highlight which level of roughage energy and concentrate is best for Holstein and Simmental cows.

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Data for this study was supplied by the project optiKuh.

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45.

Effects of continuous or pulsed-feeding application of a multi-strain probiotic on body weight gain in whiteleg shrimp *(Litopenaeus vannamei)*

Effekte einer kontinuierlichen oder gepulsten Fütterung eines Mehrstamm-Probiotikums auf die Körpergewichtsentwicklung von Garnelen (Litopenaeus vannamei)

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Pathogens and challenging conditions in shrimp farming are common problems. Unfavorable conditions affect growth, whereas probiotics support the gut health of shrimp, thereby improve performance and efficiency in production. The aim of the present study was to compare a continuous application over 12 weeks and three different alternating application protocols of a multi-species (*Bacillus sp, Enterococcus sp, Lactobacillus sp, Pediococcus sp*) probiotic feed supplement (AquaStar® Growout, Biomin GmbH, Austria) on growth performance in whiteleg shrimp.

Methods: Whiteleg shrimp (1.1 ± 0.01 g) were stocked in a RAS tank system at a density of 15 shrimp / 100 L and fed with a commercial diet (Uni President V991, 40% crude protein) to meet the requirments according to their body weight for a 12-week period. AquaStar® Growout was applied to the feed by top dressing with oil as a carrier. The dry pellets were kept in vacuum bags and stored under 18˚C, protected from light and air. In order to guarantee the quality of the feed and avoid rancification due to oil oxidation, feed was prepared freshly every two weeks. The animals were randomly assigned to the following groups and were fed accordingly: **C**: continuously fed control feed without supplement, **T1**: continuous application of 3g/kg AquaStar® Growout, **T2:** alternating application of 3g/kg AquaStar® Growout for one week followed by one week control feed, **T3:** alternating application of 3g/kg AquaStar® Growout for two weeks followed by two weeks control feed, **T4**: alternating application of 3g/kg AquaStar® Growout for three weeks followed by one week control feed. During the entire experiment the water temperature was 31.1 ± 0.8 °C, dissolved oxygen 7.23 ± 0.9 ppm, pH 8.12 ± 0.1 and nitrogen parameters water quality were total ammonia-nitrogen (TAN) 0 ppm, nitrites < 0.5 ppm and alkalinity > 150 ppm. Feeding rate was regulated to minimize the quantity of uneaten feed but remained as consistently as possible during the experiment. Uneaten feed was removed from tank. Animals were fed 6 times per day (around the clock) until satiety and feed consumption was recorded daily. Animals were weighed at the beginning and the end of the trial**.** Data was statsiticall analysed using the programme R 3.5.0 (ANOVA, with subsequent adhoc comparisons, P< 0.05).

Results: Irrespective of the feeding regime, AquaStar® Growout significantly improved growth and feed conversion rate. No effect was recorded on survival rate.

Conclusion: Data from the present study indicate that effects are most prominent when AquaStar® Growout was fed either continuously or according to T4, where a 3-week application was interrupted no longer than one week.

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46.

Gastric health in fattening pigs fed fermented liquid diets ─ findings on diets and at slaughterhouses

Einfluss von fermentiertem Flüssigfutter auf die Magengesundheit von Mastschweinen ─ Befunde zum Futter und am Schlachtof

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Introduction: Fermentation (during ~ 24 h before feeding) is a liquid feeding concept, which is increasingly widespread in German pork production. Recent studies indicate that fermentation could be accompanied by a loss of 'structure' [1]. Under standardized conditions in the institute, GRONE et al. (2018) emphasized that supplements of coarse components to the fermented liquid feed (FLF) are necessary to maintain gastric health [2]. The aim of the study was to evaluate the effect of partly fermented diets on gastric health in slaughtered pigs from three fattening farms.

Material and methods: The three farms were located in north-west Germany. All of them fed FLF to their pigs. The share of FLF in the whole diets as well as the kind of (coarse) cereals which were added to the FLF after fermentation differed between the farms. On farm A ~ 50 % of the diet were fermented (rye and rapeseed extracted meal) and the remaining 50 % consisted of unfermented components (mainly rye and wheat; commonly ground by hammer mill). The diet on farm B consisted of ~ 40 % FLF (rye, wheat, soybean and rapeseed extracted meal) and in the unfermented fraction rolled cereals were added (17.5 % of the whole diet: rolled wheat). On farm C the proportion of FLF in the diet varied at ~ 40 % and almost 50 % of the whole diet consisted of unfermented coarse triticale (ground by hammer mill, 5 – 6 mm sieve inset). Wet sieve analyses were done repeatedly to characterise the particle size distribution in the FLF as well as in the whole diets. The gastric health was evaluated by scoring stomachs of slaughtered pigs from the three farms. Therefore alterations in the pars nonglandularis were scored (0 = no changes, 1 = slight-, 2 = moderate-, 3 = high hyperkeratosis, 4 = erosion, 5 = ulcer). 24 stomachs from farm A as well as 24 from farm B and 23 from farm C were investigated. Statistical analyses were done by using SAS® software (Mean, SD and Kruskal-Wallis-test with post-hoc Dunns-test).

Results: The proportion of fine particles (particle size < 0.2 mm) in the FLF of each farm exceeded the recommended level of 35 % (proportions of particles < 0.2 mm in the FLF: 60 – 70 %). The addition of coarse particles on farm B and C decreased the proportion of fines (48.7 % vs. 47.7 %) and increased the share of particles > 2.0 mm (17.3 % vs. 15. 4 %), whereas on farm A still 57.5 % were < 0.2 mm and just 8.08 % > 2.0 mm. The score-values of stomachs from farm B and C (Score: 1.13 ± 1.44 and 1.61 ± 1.39) were significantly lower than on farm A (Score: 3.06 ± 1.48). Severe lesions (Score 4 and 5) occurred in 50 % of all investigated individuals from farm A, whereas this percentage was markedly lower in pigs from farm B (16.7 %) and C (13.0 %).

Conclusion: The present study supports the conclusions of GRONE et al. (2018). Although the diets differed in botanical and chemical compositions between the farms, an addition of coarse feed materials to the FLF had marked effects on gastric health. Data from slaughterhouse (in the field) confirm the changes in physical form / loss of coarse particles and their predisposing role for the development of gastric ulcers. But the addition of rolled cereals or cereals ground coarsely by hammer mill (5 – 6 mm sieve inset) can avoid the development of severe alterations when using FLF and enables to have the benefits of FLF (gut health, protein- / phosphorus digestibility ↑ and *Salmonella* ↓).

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47.

Effects of feeding chopped hay and grass-clover-silage on feed selection behavior and chewing activity in organic dairy cows

Einfluss von vermahlenem Heu und nachzerkleinerter Kleegrassilage auf das Futterselektionsverhalten und die Kauaktivität von Milchkühen unter ökologischen Bedingungen

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Particle size of barn-dried hay and grass silage, both commonly used in organic dairy rations, can be very long, especially when using balers or loading wagons for harvesting. This makes it difficult to formulate uniform TMR and increases sorting behavior of cows. Reduced forage particle size (RFPS) improves uniformity, thus reducing cows' sorting behavior (1), and facilitates feed comminution. This may improve intake of forage NDF, but the effects on rumination are difficult to predict. While longer particles are needed for rumination, less sorting behavior might stimulate rumination too. Therefore, the aim of this research was to evaluate the effects of a forage based TMR (80% of DM) with RFPS on sorting behavior, eating, and rumination activity.

Methods: 20 Holstein cows were balanced by average milk yield (26.7 kg), body weight (703 kg), days in milk (135d), and number of lactations (3.8) into 2 feeding groups. CALAN gates were used to record individual feeding intake. After a covariate period (7d), groups were switched to a TMR, either with a conventional particle size (CON) or RFPS (RED) for 34d, including an adaptation period of 12d. TMR contained equal proportions (on DM basis) of hay (43%), grass-clover-silage (37%), and concentrate (20%), resulting in 6.92 MJ NE_L, 151 g CP, 35g starch, and 437g NDF per kg DM. Before preparing the RED ration, hay and silage were chopped at theoretical length of cut of 0.5 cm and hay additionally hammer-milled (2 cm sieve). DM content (44%) of both rations was adjusted with water. TMR were offered twice daily (06:00, 17:00), allowing for 10% feed refusals. Particle size distribution (PSD) was determined using 3 screens (19 mm, 8 mm, 1.18mm) and a pan. Samples of fresh TMR and each cows' refusals (collected twice daily) were sieved on 2 × 4 consecutive days and on 2d during the covariate period. Sorting behavior (selection indices) was evaluated based on the difference in PSD of fresh feed and refusals according to (2). A selection index equal to 1 indicates no sorting, while values >1 indicate a selection for a certain particle fraction and vice versa. Each cow's chewing activity was measured with RumiWatch halters (using the converter V0.7.4.13) for 7 consecutive days and for 3d in the covariate period. Statistical analysis was carried out with proc mixed (SAS 9.4) considering effects of the feeding group, day, and covariates for the corresponding parameter, and cow nested within the group was considered a random effect.

Results: Range of PSD accounted for 68.5 and 23% points in CON and RED, respectively. Range of selection indices was lower in RED (0.05) than in CON (0.10), indicating a less pronounced sorting behavior in RED-fed cows. Intrestingly, all cows preferred particles >19 mm and cows on the RED diet selected rather for particles <19mm than cows on CON. Although significantly reduced, total chewing time of RED-fed cows (853 min/d) was still high. RED cows' eating time decreased ($P<0.001$) by 69.3 min/d, while ruminating time tended ($P=0.069$) to increase by 24.6 min/d. As DMI of the RED diet was increased ($P<0.001$) (+1.8kg/d), it also resulted in a higher forage NDF intake.

Conclusions: Including hammer-milled hay and re-chopped silage offered cows a more uniform diet, reduced sorting behavior, and reduced mastication efforts during eating, but not rumination. The finding that all cows showed a preference for long particles was unexpected and remains unclear. The slight increase in rumination time suggests that the physical properties of the RED ration still fulfilled the requirements of dairy cows in this study.

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The study was funded by the BMNT, Land Salzburg, Raiffeisen Salzburg, and the "Ja! Natürlich" brand of the REWE Group.

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48.

Effect of an energy- and nutrient-reduced diet containing 10% lignocellulose on the development of gastrointestinal organs, intestinal histomorphology and intestinal metabolites in dual purpose laying hens

Effekte einer energie- und nährstoffreduzierten, 10 % Lignocellulose enthaltenden Diät auf die gastrointestinale Organentwicklung, intestinale Histomorphometrie und intestinale Metabolite bei Legehennen einer Zweinutzungslinie

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Dual purpose laying hens fed 10 % dietary lignocellulose showed a lower body fat content and an increased laying performance compared to hens fed a standard layer diet (1). Lignocellulose is a low fermentable polymer, acting as a diet diluent and may influence physiological and digestive processes. Studies have shown that the dietary supplementation of lignocellulose at low levels might have an impact on intestinal microbiota and microbial metabolic activity in broilers (2). Thus, the present study investigated the effect of a 10 % dietary lignocellulose dilution on the development of gastrointestinal organs, intestinal morphology and intestinal metabolites in dual purpose laying hens.

Methods: One-day-old female Lohmann Dual chicks were allocated to 12 pens and fed two different diets for a period of 52 weeks: A standard control diet (C) and a treatment diet (LC), based on the control diet but diluted with 10% lignocellulose (Arbocel®, J. Rettenmaier & Söhne GmbH + Co KG, Germany). At 52 weeks of age, hens were killed, gastrointestinal organ weights determined and rectal tissue samples fixed in formalin and stained with hematoxylin and eosin for histomorphological examinations. Caecal digesta samples were analysed for ammonia- and lactic acid concentrations (photometry and high performance liquid chromatography) as well as for the concentration of short-chain fatty acids (SCFAs) (gas chromatographic). Statistical analyses included Students t test and Pearson correlation analyses (SPSS 25.0, Chicago, IL). Differences at $P < 0.05$ were considered significant.

Results: The results showed that the relative masses of gastrointestinal organs were affected by feeding the different diets. The relative organ masses of the gizzard, small and large intestine were increased in hens fed LC compared to those fed C ($P < 0.05$). Results of the histomorphological examinations showed that hens fed LC had an increased rectal villus area and surface area as well as an increased rectal crypt surface area than hens fed C ($P < 0.05$). The total amount of SCFAs in the caecum in particular, the concentration of acetic acid, propionic acid and n-valeric acid, and the amount of ammonia were higher in C fed hens compared to LC fed hens ($P < 0.05$). Correlation analyses revealed a negative relationship between the concentration of SCFAs in the caecum and the rectal villus and crypt surface area ($P < 0.01$).

Conclusion: In conclusion, this study shows that dietary lignocellulose increased the relative masses of gastrointestinal organs, which was accompanied with the development of a higher rectal mucosal surface area. The amount of SCFAs was reduced in LC fed hens underlining the bird's low capacity to ferment lignocellulose by microbes in the hindgut. Interestingly, the concentration of SCFAs in the caecum of hens was negatively correlated with the rectal villus surface area, which might indicate a compensatory reaction of birds fed LC enhancing the absorption of bacterial metabolites by increasing the intestinal mucosal surface.

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49.

Preliminary test in piglets offered a compound feed containing humic acid-rich peat on performance, fermentation characteristics and composition of the microbiome in the digesta

Taststudie zum Einfluss Huminsäure-reichen Torfes als Mischfutterkomponente für Ferkel auf die Leistung, die Fermentationscharakteristika und die Zusammensetzung des Mikrobioms im Chymus

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Extensive research on the specific use of feed ingredients has been undertaken to control the period following weaning characterized by a high incidence diarrhea and depression of growth performance in piglets. Humic substances may alter microbiota in the intestinal digesta and therefore support animal health.

Methods: The tests were carried out with 15 weaned piglets, divided into three groups. From day 28 onwards, an experimental diet consisting of wheat, barley, soybean meal, wheat bran, oat flour, confectionary products, wheat flour, macerated wheat, beet pulp, sunflower seed extract, calcareous lime, fish concentrate, plant oil, monocalciumphosphate, sodium chloride, plant fatty acid, fish oil, sodium bicarbonate and feed additives was offered to the piglets (Ctr [0% peat]; H1.5 [1.5% peat] and H3.0 [3.0% peat]; all approx. 178 g CP, 13.7 MJ, 13.3 g Lys, as fed) for 28 days . Performance parameters were recorded. At necropsy, contents of caecal and colonic digesta were removed. Parts of this material were used to measure fermentation products (n = 5 per group) and gas formation using an in vitro batch test with specific fermenters (n=3 per groups). Exactly 60 ml of liquid chyme were incubated with 27 ml of a starch solution (4.44 g starch powder in 100 ml physiological isotonic buffer solution) in a water bath at 40°C (minimum in duplicate). Gas production was measured over 4 h. For microbiome studies 16S rRNA amplification was performed within the hypervariable region V 4 and sequenced with Illumina-Miseq platform. DNA reads processing and statistical analysis was performed using QIIME (version 1.8.0), MicrobiomeAnalyst, RStudio and SAS Enterprise Guide.

Results: The mean body weight of the animals at the end of the experimental period differed only numerically between groups (in kg; start: Ctr: 8.93±0.92, H1.5: 8.96±0.54, H3.0: 8.84±1.70, final: Ctr: 26.1±4.85, H1.5: 28.5±3.41, H3.0: 26.2±4.92). On average, animals of group "H1.5" were about 2 kg heavier at dissection. The daily weight gains were high for this age (in g/day; day 30-58: Ctr: 607±157; H1.5: 692±101; H3.0: 615±113), the feed to gain ratio extremely low (in g/g; day 30-58: Ctr: 1.538; H1.5: 1.462; H3.0: 1.462). Concentrations of short-chain fatty acids in the caecal content were significantly lower when peat was used (mmol/kg wet weight; Ctr: 173±30.0; H1.5:134±15.0; H3.0:133±17.3). There was no significant effect on gas formation. Compared to the control group, the gas formation in the peat groups was numerically reduced (in mL gas per 10 mL batch in 4 h; Ctr: 7.9±2.2; H1.5: 7.4±2.4; H3.0: 6.6±1.1). With regard to the microbiome analysis in caecal contents, the Mann-Whitney/Kruskal-Wallis test for univariate statistical comparisons between the diet groups showed significant differences for relative abundance in *Tenericutes* at phylum level and *Mollicutes* at class level ($p < 0.05$). The microbiome analyses in the cecal content showed significantly higher values for alpha diversity Chao 1 index for samples from the control group.

Conclusions: The use of a humin acid-rich peat product in an isoenergetic and isonitrogenic diet with constant amino acid supply had a rather positive effect on the overall performance of healthy pigs. According to our initial findings, peat influences intestinal microflora causing a shift in the overall concentration of fermentation products pattern. Therefore, peat is rather not an "inert substance" within a diet, but an organic compound that affects the microbiome.

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50.

Effects of marginal differences in dietary physically effective fiber concentration on feed intake and feeding behavior in lactating dairy cows

Einfluss von marginalen Differenzen des physikalisch effektiven Fasergehalts im Futter auf Futteraufnahme und Fressverhalten von laktierenden Milchkühen

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The aim of the study was to investigate the linear and quadratic effects of graded physically effective neutral detergent fiber (peNDF) concentrations of a total mixed ration (TMR) by varying particle size (PS) on feed intake and feeding behavior of dairy cows. It was hypothesized that increasing dietary peNDF concentration increases rumination activity, but may decrease feed intake and therefore rumination at very low and very high dietary peNDF concentrations.

Methods: Eight lactating German Holstein cows (arithmetic mean ± standard deviation: 197 ± 73 d in milk, 41.3 ± 6.5 kg milk/d) were assigned to a 4 x 3 Youden square with three 8-d periods (4 d adaptation, 4 d sampling). Animals were grouped to equalize mean days in milk, milk production, and dry matter (DM) intake. A TMR with maize silage, grass silage, and a concentrate mixture at a forage to concentrate ratio of 59:41 on DM basis was prepared every morning and offered *ad libitum*. To avoid confounding effects of dissimilar fiber concentrations between diets, solely dietary PS was varied by increasing TMR mixing times using a modified mixer wagon (Power Champ L, Marmix GmbH & Co.KG, Unterwachingen, Germany) to create four diets varying in mixing time (D_1-D_4): D_1 (28 min), D_2 (43 min), D_3 (58 min), and D_4 (73 min). Feed samples were analyzed for DM and neutral detergent fiber. Dietary PS distributions were determined using the Penn State Particle Separator with three sieves (19, 8, and 4 mm) and used to calculate dietary peNDF. An automatic weighing trough system registered feeding time, trough visits, and fresh matter intake of individual animals. Chewing behavior of individual cows was recorded using noseband pressure sensors (RumiWatch, Itin+Hoch Gmbh, Liestal, Switzerland). Data was analyzed using PROC MIXED in SAS V9.4 with diet and period as main effects and the interaction between animal and group as random factor. Linear and quadratic effects were determined using polynomial contrasts tests and declared significant at $P<0.05$.

Results: Increasing mixing time of TMR linearly decreased dietary concentrations of $peNDF_4$ (particles > 4 mm) from 30.1 to 26.8 g/100 g DM ($P<0.01$) and $peNDF_8$ (particles > 8 mm) from 26.4 to 22.1 g/100 g DM ($P<0.01$). Dietary peNDF concentration did not significantly affect DM intake ($P=0.41$), which averaged 25.8, 26.3, 26.5, and 25.6 kg/d for D_1, D_2, D_3, and D_4, respectively. Increasing peNDF concentration in the diet linearly increased daily eating time from 257 to 331 min ($P=0.02$) and numbers of eating chews (in n/d and n/kg DM intake; $P\leq0.01$). Similarly, trough visiting time increased linearly with increasing dietary peNDF concentration from 3.3 to 4.1 h/d ($P<0.01$). As also number of trough visits increased from 35 to 45 visits/d ($P<0.01$), time spent in the trough per visit was similar across all treatments ($P=0.08$). No effects were observed for daily rumination time ($P=0.18$) nor rumination chews (in n/d and n/kg DM intake; $P\geq0.18$).

Conclusions: Increasing dietary peNDF concentration by varying PS does not affect feed intake and rumination activity of dairy cows. However, increasing peNDF concentration of the diet increases daily number of trough visits. Such more equal distribution of the animal's nutrient intake throughout the day could potentially contribute to a more stable rumen pH and thus improved rumen health in dairy cows.

This study was supported by H. Wilhelm Schaumann Stiftung.

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51.

Survival rate of *Salmonella Typhimurium* in a liquid diet for pigs simulating the 'controlled' fermentation

Überlebensrate von Salmonella Typhimurium im Flüssigfutter für Schweine bei Simulation der „kontrollierten" Fermentation

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Introduction: 'Controlled' fermentation is a liquid feeding concept, which promises to favor gut health and to reduce the prevalence of *Salmonella* in pork production by high contents of lactic acid bacteria and lactic acid in the liquid diet [1]. The aim of the study was to evaluate *in vitro* the impact of feed fermentation on the survival of *Salmonella* Typhimurium after experimental inoculation in a liquid diet (= 'hygienising' potential of the fermentation process).

Material and methods: The fermentation process was simulated in 100 ml bottles (*Duran® Original Laborflasche, GL 45, 100 ml, DWK Life Sciences GmbH, Wertheim*), which were equipped with a magnetic stir and filled with a liquid diet consisting of (%) rye 50, rapeseed extracted meal 30, wheat 10 and barley 10 (feed : water ratio 1 : 3.2). The bottles contained 37 g fresh matter of this liquid diet. After filling the liquid diet was experimentally inoculated with a field isolate of *S.* Typhimurium (isolated from faeces of fattening pigs). The fermentation process was started by adding a freeze-dried, granulated starter culture (*SchaumaLac Feed Protect XP Konzentrat, H. Wilhelm Schaumann GmbH, Pinneberg*) at the beginning of fermentation in a dosage of 2 x 10^5 cfu/g liquid diet. The bottle (= fermenter) was put on a magnetic stire (*MIXdrive 15, 2mag AG, Munich*) in an incubator to ensure 200 rotations per minute (fermentation temperature: 38 °C), whereas the control bottle (no addition of starter culture) remained at room temperature (~ 20 °C).

Immediately before inoculation a cultural investigation (*Thermo Scientific™ Oxoid™ Brilliance Salmonella Agar-Basis, Co. Oxoid, Wesel*) was done to guarantee a *Salmonella* free status before the experimental inoculation. The counts of Salmonella in the liquid diets were also culturally determined directly after inoculation and 4 h, 12 h and 24 h after inoculation. The procedure was replicated three times (n = 3/treatment). Statistical analyses were done by using SAS® software (Mean, SD and t-test).

Results: As expected no *Salmonella* were found in the liquid diets before the experimental inoculation. Immediately after inoculation 7 $\log_{10}$ cfu/g liquid diet of *S.* Typhimurium were detected in both approaches (control / fermentation). In both types of the liquid diet the counts of *S.* Typhimurium increased 12 h after inoculation to 8 $\log_{10}$ cfu/g. But after 24 h the counts differed significantly between the control and fermentation approach: In the fermented liquid feed (pH: 3.81 ± 0.055) no *Salmonella* were culturally verifiable (< 2.00 ± 0.000 $\log_{10}$ cfu/g), whereas in the control approach (feed pH: 5.12 ± 0.082) still high counts were found (8.80 ± 0.055 $\log_{10}$ cfu/g).

Conclusion: Contaminated feed is an entry way for *Salmonella* into pig farms. In Germany the risk of feed being contaminated with *Salmonella* is in general relative low [2], but in the past *Salmonella* was found i. a. in extracted meals not seldom [3]. The present *in vitro* study demonstrates the enormous 'hygienising' effects of feed fermentation and the potential of fermented liquid feed to reduce *Salmonella* prevalence in pigs. Liquid diets without controlled fermentation (stored at 20° C) even offer chances for *Salmonella* growth, whereas the process of controlled fermentation reduced the counts strongly.

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52.

Growth, milk intake, and plasma metabolites and free amino acid concentrations in low and normal birth weight piglets in the late neonatal period

Wachstumsparameter, Milchaufnahme und Plasmakonzentrationen von Metaboliten und freien Aminosäuren bei neonatalen Ferkeln mit niedrigem und normalem Geburtsgewicht

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Low birth weight (LBW) has been shown to negatively impact the growth and health of piglets before and after weaning (1). To date combined data on growth and plasma metabolic profiles during the neonatal period in LBW and normal birth weight (NBW) piglets are scarce. The objective was to investigate the association of birth weight with milk intake, growth parameters, plasma metabolite and free amino acid (AA) concentrations, in the late neonatal period. We hypothesize that LBW is associated with alterations in plasma metabolic and AA profiles.

Methods: At birth, 12 pairs of male German Landrace littermates born to first parity sows were selected and suckled by their dams for 12 days, in standardized litters of 12 piglets/sow. Each pair had one LBW (1.11 kg ± 0.03, n=12) and one NBW (1.50 kg ± 0.04, n=12) piglet. Piglet body weight was measured daily. Crown-rump length, abdominal circumference and rectal temperature were measured at birth (day 0), 7 and 12 days postnatal (dpn). At 11 dpn piglets were injected with 70% D_20 (*i.p.* 1 g /kg BW) and milk intake was calculated by isotope dilution (2). Plasma was collected 4 h after birth and 11 dpn via venipuncture, and at 12 dpn via cardiac puncture, to analyse milk intake, plasma metabolites and free AA concentrations. Data was analysed using the MIXED procedure of SAS, and where applicable, with repeated measures. Least square means were separated using the Tukey test ($P<0.05$).

Results: From birth until 12 dpn, LBW piglets were lighter ($P<0.01$) and had reduced ($P<0.01$) average daily gain (g/day) compared with NBW piglets. Crown-rump length ($P<0.05$; birth = 8.8%, $P<0.01$; 12 dpn = 8.2%), abdominal circumference ($P<0.05$; birth = 10.8%, 7 dpn = 10.6%, 12 dpn = 10.5%) and body mass index (kg/m^2: birth, $P<0.10$; 11.5%, 7 dpn, $P=0.01$; 12%), ponderal index (kg/m^3: 7 dpn, $P<0.05$; 12.4%,) were smaller in LBW compared with NBW piglets. Milk intake at 12 dpn was higher in LBW compared with NBW piglets (328 vs 296 g/kg BW/day) ($P<0.05$). At 4 h after birth, concentration of plasma inositol was higher (3.7 vs 2.8 µmol/L; $P<0.01$) and non-esterified fatty acids (NEFA, 186 vs 243 µmol/L; $P<0.05$), albumin (7.9 vs 9.1 g/L; $P<0.05$), histidine (143 vs 166 µmol/L; $P<0.05$), and glucose were lower (5.2 vs 7.1 mmol/L; $P<0.05$) in LBW piglets compared with NBW piglets. At 12 dpn, the plasma concentrations of total AA, essential AA, the ketogenic AA family, leucine ($P<0.001$), asparagine, isoleucine, valine ($P<0.01$), glucogenic AA family, histidine, lysine, methionine, and serine ($P<0.05$) were higher in LBW compared with NBW piglets.

Conclusions: At 4 h after birth, lower concentrations of glucose, albumin, NEFAs and histidine suggests reduced colostrum intake by LBW piglets, and higher plasma inositol suggests altered glucose metabolism (3). Higher milk intake and increased plasma AA concentrations at 12 dpn might indicate that LBW piglets were unable to fully utilise the available pool of AA, suggesting reduced tissue protein synthesis and/or energy deposition.

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53.

Body weight gaining - Impact on metabolic health in ponies and horses

Körpergewichtszunahme – Einfluss auf die metabolische Gesundheit von Ponys und Pferden

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Equine obesity is accompanied by metabolic changes and diseases. For example, equine metabolic syndrome (EMS) is a challenging health issue. Data in obese horse showed an increase in insulin concentrations with excessive energy intake but changes in non-esterified fatty acid (NEFA) are still poorly studied. The aim of this study was to illuminate changes in the metabolic profile with progressive body weight (BW) gain in ponies and horses.

Methods: 19 lean and metabolic healthy animals (10 Shetland ponies, 9 warmblood horses) were included in the study. Animals were housed in box stalls and had free access to a sand paddock for 5 hours a day. Over two years equines received 200 % of their maintenance energy requirements (metabolizable energy $MJ/kg^{0.75}$; energy intake covered by hay (60 %) and compound feed (40 %)). At three sampling points (t0 = beginning; t1 = after one year; t2 = after two years of excessive energy intake) blood was collected after an overnight fast and an intravenous combined glucose insulin test (CGIT) was performed. Plasma glucose, serum insulin, serum NEFA and serum triacylglyceride (TG) concentrations were measured according to validated assays. Data are expressed as mean ± standard deviation. An ANOVA with repeated measurements was performed. The LSD test was applied when indicated. The project was approved by the Ethics Committee (No. TVV 32/15) and funded by the German research foundation (VE 225/9-1).

Results: Equines increased BW in the first year ($P < 0.001$) but BW remained constant in the second year of excessive energy intake. Horses had hay leftovers, but energy intake still exceeded energy requirements by 74 ± 7.18 %. In contrast to the horses, ponies had no leftovers. In ponies, basal plasma glucose (t0: 3.53 ± 0.636 mmol/L; t2: 4.34 ± 0.861 mmol/L; $P = 0.001$), serum insulin (t0: 4.26 ± 1.36 µU/mL; t2: 13.9 ± 14.9 µU/L; $P = 0.019$) and serum NEFA (t0: 119 ± 117 µmol/L; t2: 352 ± 141 µmol/L; $P = 0.01$) increased significantly comparing t2 to t0. In horses, serum insulin increased significantly (t0: 8.96 ± 8.31 µU/L; t2: 15.1 ± 10.3 µU/L; $P = 0.024$) with BW gaining. Plasma glucose and serum NEFA levels did not change in horses over the BW gaining period. No differences were found concerning serum TG concentrations in both breeds. During CGIT serum NEFA levels showed a drop after the initial insulin bolus. 150 minutes after insulin application serum NEFA concentrations reached basal levels. In ponies the drop in serum NEFA levels was greater at t2 than at t0.

Conclusion: Over two years of excessive energy intake, basal plasma glucose and serum NEFA increased in ponies but these parameters remained constant in horses. The NEFA concentration curve during CGIT changed only in ponies with BW gain. These results may give a clue for the higher susceptibility of ponies for metabolic dysregulations linked to obesity.

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54.

Effects of equine obesity on liver metabolism

Auswirkungen equiner Adipositas auf den Leber-Metabolismus

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The impact of equine obesity on liver metabolism has not been intensively investigated yet, even though the non-alcoholic fatty liver disease (NAFLD) is a confirmed component in human obesity as part of the human metabolic syndrome. NAFLD is associated with elevated serum liver enzymes and altered hepatic cytokine mRNA expression. Furthermore, the reasons for the higher predisposition of ponies for metabolic disorders under obese conditions have not been identified yet. Therefore, the aim of the study was to investigate hepatic alterations with increasing body weight (BW) in ponies and horses. M**ethods:** 10 Shetland ponies (age 6 ± 3 years, mean BW ± SD: 118 ± 29 kg) and 9 Warmblood horses (age 10 ± 3 years, mean BW ± SD: 602 ± 45 kg) were included in the study. According to a body condition score (BCS) on a scale from 0 to 5 all animals started in a non-obese condition. In two subsequent years all animals received 200% of their maintenance energy requirements. BW and BCS were monitored weekly. Blood samples were collected for assessment of serum liver enzyme activities (ALP, AST, GGT, GLDH and bile acids) in yearly intervals. Simultaneously, liver tissue was taken under general anaesthesia 15 hours after a lipopolysaccharide challenge for RT-qPCR analysis of inflammatory markers (IL-6, IL-1β, TNFα, CD68, NF-κB, chemerin). The project was approved by the Ethics Committee for Animal Rights Protection of the Leipzig District Government (No. TVV 32/15). Data were assessed for normality by the Shapiro-Wilk test. Serum liver enzymes, bile acids and hepatic mRNA levels were analysed by Friedman ANOVA. Statistical significance was accepted at $P<0.05$. **Results:** Within two years, mean BW increased 29.9 ± 18.4% in ponies and 16.2 ± 6.3% in horses. Ponies started with a median BCS of 3.7 and horses with 3.6 score points. Ponies and horses reached a median BCS of 4.8 after two years of BW gain. Serum GLDH and bile acids increased significantly with increasing BW in ponies but not in horses. mRNA levels of chemerin increased significantly with BW gain in ponies and in horses. The remaining parameters showed only minor changes. **Conclusion:** We detected differences in the liver metabolism under obese conditions comparing horses and ponies. This might be one reason for the higher predisposition of pony breeds for metabolic diseases. Furthermore, chemerin might become a useful tool, investigating equine obesity. Further studies are needed.

This study is funded by the German Research Foundation (DFG, VE 225/9-1).

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55.

Impact of inulin/oligofructose supplementation to a high-protein or control diet on body weight gain and intermediary metabolism in mice

Einfluss einer Inulin/Oligofructose Supplementierung zu einer Hochprotein- oder Kontrolldiät auf die Gewichtsentwicklung und den Intermediärmetabolismus von Mäusen

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Prebiotics such as inulin and oligofructose (FOS) are non-digestible nutritional compounds, which increase gastric filling, intestinal motility and short-chain fatty acid (SCFA) production. FOS supplementation to a high-fat but not control diet prevented body weight gain and exerted positive effects on insulin resistance and lipid metabolism (1). However, it is not known how inulin/FOS supplementation affects the intermediary metabolism on a high-protein diet background. Thus, the aim of this study was to investigate if the response of inulin/FOS supplementation to a high-protein diet differs from isoenergetic control diet and alters feed intake, body weight gain, digesta transit time and energy metabolism in mice. **Methods:** Forty 5-weeks-old male C57BL/6NCrl mice were housed individually at 22°C, with a 12:12-h light-dark cycle and *ad libitum* feeding. Mice received a control diet (CD; 16.2 MJ/kg gross energy) for 2 weeks. At the age of 7 weeks, each 10 mice were randomly allocated to one of four experimental diets: CD and high-protein diet (HP; 40% protein; 16.0 MJ/kg) without and with 10% inulin/oligofructose (1:1) supplementation (CD+I and HP+I) for 4 weeks. After 3 weeks on the experimental diets, indirect calorimetry was performed in respiration chambers for 48 h to measure total energy expenditure (TEE; kJ/d), physical activity, carbohydrate (COX), fat oxidation (FOX), and fecal nitrogen excretion. After 4 weeks, blood was taken from the tail and fasted glucose concentrations were measured by a glucometer. Total gastrointestinal transit time was measured by the gavage of 120 µl carmine red (10 mg/ml), and time for expulsion of the first red fecal pellet was determined. Data analysis were performed by ANOVA using the MIXED procedure (Tukey test) of SAS (Version 9.4), for repeated measurements including the fixed effects diet and, FOSand if applicable time, as well as their interaction diet x FOS, and if applicable diet x time, FOS x time and FOS x diet x time. **Results:** The feed intake was not different between diets. However, at the first two days of the trial, energy intake was significantly lower in CD+I compared to CD ($P<0.05$), whereas the overall energy intake over the 4 week period did not alter between diets and inulin/FOS supplementation. After 3 weeks on the experimental diets, body weight gain of CD and HP+I was higher than in HP mice ($P<0.05$, respectively). Blood glucose concentration was significant higher in HP+I ($P<0.05$) and in CD than in HP mice ($P<0.01$). Indirect calorimetry revealed higher COX per unit mBW in CD+I than HP+I mice ($P<0.05$) and as a trend in HP compared to HP+I ($P=0.1$), whereas these differences were not evident when the results were normalized to energy intake. The TEE was lower in HP+I than HP ($P<0.05$) and CD+I mice ($P<0.01$), whilst the physical activity was higher in HP than CD and HP+I mice ($P<0.01$, respectively). The fecal nitrogen excretion tended to be lower in HP+I ($P=0.06$) and was significant lower in CD than HP mice ($P<0.05$), however, this effect was not apparent when normalized to nitrogen intake. Total nitrogen excretion, body weight gain, fasted blood glucose concentration and physical activity was not different between CD, CD+I and HP+I mice. Inulin/FOS supplementation to CD did not change TEE and COX per unit mBW. FOX and the mean digesta transit time did not differ between dietary groups. **Conclusion:** Inulin/FOS supplementation compensates for HP diet-induced effects on body weight gain, fasted blood glucose, physical activity and nitrogen excretion and reaches CD levels. These results suggest that supplementation of inulin/FOS to a HP but not CD spares glucose but not fat utilization, which together with lower physical activity and TEE increases body weight gain. The effect of FOS only under HP but not CD conditions may be due to the low starch content of the HP diet.

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56.

Increase in hypothalamic microglia activation during early lactation of dairy cows

Erhöhte hypothalamische Mikroglia-Aktivierung im Zeitraum der Frühlaktation der Milchkuh

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During the transition from late pregnancy to early lactation, dairy cows enter into negative energy balance associated with increased physiological imbalance (PI) (1) and reduced immunity. At this time, cows are at increased risk of diseases such as mastitis, metritis or ruminitis, all characterized by elevated plasma concentrations of lipopolysaccharide (LPS), pro-inflammatory cytokines, and reactive oxygen species. Peripheral LPS administration results in hypothalamic inflammation as indicated by an increased number of activated microglia, which in turn may affect the level of feed intake (2). The aim of the present study was to investigate whether hypothalamic microglia activation is increased during early lactation of dairy cows by using the microglia-specific marker allograft inflammatory factor 1 (AIF1).

Material and Methods: Late and early lactating cows were fed ad libitum a total mixed ration consisting of grass silage, corn silage, hay, straw and concentrate according to their requirements. Hypothalami and parallel blood samples were obtained from 5 non-pregnant, late-lactating Holstein dairy cows (180-325 days in milk, 2nd- 4th lactation) and 10 early-lactating cows (11-66 days in milk, 3rd and 4th lactation). Brain tissue was fixed in formaldehyde, embedded in paraffin and cut in 4 µm slices. After blocking, activated microglia was immunostained for AIF1 and nuclei were counter-stained with haematoxylin. Plasma was analysed for glucose, beta-hydroxybutyrate and non-esterified fatty acids (NEFA) concentrations using commercial enzymatic kits to calculate PI (1). The plasma fatty acid profile was analysed by gas chromatography/flame ionization detection (GC/FID). Statistical analysis was performed using a t-test and Pearson correlation.

Results: The number of activated microglia cells in the periventricular region of the 3rd brain ventricle was 2.8-times higher in early than late lactating cows ($P<0.05$). There was a strong positive correlation between the total number of AIF1-positive cells and the maximal distance of a positive cell from the border of the 3rd ventricle ($P<0.01$). In hypothalamic slices from late lactating cows, AIF1-positive cells were predominantly found in short distance from the 3rd ventricle border, whereas in early lactating cows, the maximal distance was much greater. While the number of activated microglia cells was negatively correlated with PI ($P<0.05$), there was no relationship between AIF-1 cells and plasma concentrations of unsaturated fatty acids, the latter considered to exert antioxidant and anti-inflammatory characteristics.

Conclusion: Cows in early lactation experience hypothalamic inflammation, independently of the level of unsaturated fatty acids, but as stronger the PI, as higher the degree of central inflammation.

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57.

The effect of feeding rumen-protected niacin during the transition from late pregnancy to early lactation on hepatic transcriptome of early-lactation dairy cows

Einfluss der Fütterung von pansengeschütztem Niacin während der Transitphase auf das hepatische Transkriptom von Milchkühen während der Frühlaktation

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High dosages of niacin (NA) were found to induce antilipolytic effects via activation of the NA receptor in white adipose tissue (WAT) and thereby to an altered hepatic lipid metabolism in dairy cows. However, almost no attention has been paid to possible direct effects of NA in the liver, despite expression of NA receptor in the liver of cattle is even more abundant than in WAT. Thus, we hypothesized that administration of a high dosage of rumen-protected NA to dairy cows influences critical metabolic or signaling pathways in the liver through modulating the hepatic transcriptome. In order to identify these pathways, we performed genome-wide transcript profiling in liver biopsies obtained at d 7 postpartum (p.p.) from dairy cows which received either no (control group) or a daily rumen-protected NA dosage (NA group) from 3 wk before calving until 1 wk p.p.

Methods: A total of 22 multiparous Holstein dairy cows were assigned to two groups (control group and NA group, each n = 11) and kept in a freestall barn equipped with weighing troughs. Both groups of cows had free access to the same corn and grass silage-based antepartum (a.p.) and p.p. total mixed rations (TMR) during pregnancy and lactation, respectively, which were calculated to meet energy and protein requirements of dairy cows (GfE, 2001). In addition, cows of the NA group were given daily a pharmacological NA dosage (79 mg rumen-protected NA per kg BW) from 3 wk before expected calving until 1 wk p.p. The individual NA dosage was mixed into a 500 g pressed sugar beet pulp portion, which was supplied to each cow once daily in feed fences ensuring complete intake. Cows of the control group were given 500 g pressed sugar beet pulp without supplemented NA. Biopsies of the liver were taken on d 7 p.p. at 0900 h under local anesthesia. For microarray analysis, total RNA isolated from liver samples of n = 9 animals per group was used for hybridization to the Affymetrix GeneChip Bovine Gene 1.0 ST array. Transcriptomic data were statistically analyzed by Student's t test.

Results: Feed intake, body weight, body condition score, the extent of negative energy balance and milk yield did not differ between cows fed rumen-protected NA and control cows at wk 1 to 3 p.p. Hepatic transcript profiling revealed that a total of 487 transcripts were differentially expressed [filter criteria fold change (FC) > 1.2 or FC < -1.2, $P < 0.05$] in the liver at d 7 p.p. between cows fed NA and control cows. Substantially more transcripts were down-regulated (n = 338) than up-regulated (n = 149) by NA in the liver of cows. Gene set enrichment analysis (GSEA) for the up-regulated transcripts revealed that the most enriched gene ontology (GO) biological process terms were exclusively related to immune processes, such as leukocyte differentiation, immune system process, activation of immune response and acute inflammatory response. GSEA of the down-regulated transcripts showed that the most enriched biological process terms were related to metabolic processes, such as cellular metabolic process, small molecule metabolic process, lipid catabolic process, organic cyclic compound metabolic process, small molecule biosynthetic process and cellular lipid catabolic process.

Conclusion: Hepatic transcriptome analysis shows that rumen-protected NA induces genes which are involved mainly in immune processes including acute phase response and stress response in dairy cows at d 7 p.p. These findings indicate that supplementation of a pharmacological dosage of rumen-protected NA to dairy cows in the periparturient period may be detrimental by inducing or amplifying the systemic inflammation-like condition which is typically observed in the liver of high-yielding dairy cows in the p.p. period.

This study was financially supported by the Deutsche Forschungsgemeinschaft (Grant no. ED 70/13-1).

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58.

Influence of quercetin on DNA methylation pattern in growing pigs

Einfluss von Quercetin auf die DNA-Methylierung bei Wachsenden Schweinen

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DNA methyltransferases (DNMTs) are key elements of epigenomic changes. DNMT1 is a maintenance DNMT, while DNMT3a and 3b are *de novo* DNMTs. Mammalian DNMT1, DNMT3a, and DNMT3b can all convert 5mc (5-methylcytosine) to cytosine which will be enzymatically demethylated to 5hmC (5-hydroxymethylcytosine) by the TET (Ten-eleven translocation methylcytosine dioxygenase) enzymes. DNMT1 regulates gene expression by methylating the CpG island promoter of a gene preventing the binding of transcription factors and results in gene silencing. Initial reports have been shown that secondary plant metabolites such as quercetin might be involved in regulating DNA methylation *in vitro* but data on farm animals is still scarce. We hypothesize, that orally applied quercetin, being one of the most bioactive flavonoids can influence global DNA methylation. Therefore, the present study determined the influence of quercetin on global DNA methylation pattern in pigs.

Methods: Male hybrid (DE×DL) castrated pigs with an initial b.w of 25 ± 2.0 kg were housed individually and were assigned to either control (C, n=5) or quercetin (Q, n=5) group. Pigs were fed with standard diet for minipigs (no. 9031, Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) supplemented without or with 0.2 % quercetin (Roth, Germany) for 7 weeks. Pigs were sacrificed after 12 hours of fasting (final b.w of 62.3 ± 2.2 kg). Tissues (≈25 g) such as liver, kidney, muscle, and adipose tissue were taken and snap frozen for later use. Samples (≈1 g) were pulverized under liquid nitrogen and 30-35 mg of each sample was used in the subsequent analyses. Genomic DNA and RNA from the samples were extracted using Qiagens` FastDNA and Trizol-RNAeasy kits following manufacturers protocol, respectively. From the extracted genomic DNA, standard dot blot technique was performed to measure the global methylation and demethylation markers (5mc and 5hmc) and signals were detected under XRS imaging system (Biorad). cDNAs were constructed from the extracted RNA of each sample followed by qRT-PCR using pig-derived DNMT1, Tet-1, and ß-actin primers. Proteins from the samples were extracted using RIFA (radioimmunoprecipitation assay) buffer with protease inhibitor cocktail and protein concentrations were determined using BCA (Bicinchoninic acid assay) method. After electrophoresis, proteins were transferred onto nitrocellulose membrane followed by incubation of DNMT1 and ß-actin antibodies overnight. Detection was done under XRS imaging system using ECL prime (GE healthcare) detector. Bands were quantified densitometrically. Data were analyzed using One-Way ANOVA followed by the Bonferroni/Dunn test for multiple comparisons. Differences were considered significant at $P \leq 0.05$.

Results: Inclusion of quercetin in pig's diet did not affect weight gains during seven weeks of feeding. However, a slight difference was observed in feed conversion rate ($P \leq 0.0551$) from week 1 to 7 of feeding. Quercetin increased methylation in a tissue-specific manner. Higher 5mc (methylation marker) signals were detected in adipose and kidney tissues ($P \leq 0.05$) whereas no differences were observed in liver and muscle tissues. Furthermore, demethylation marker (5hmc) were also higher in adipose and kidney indicating the rapid demethylating process. In concordance to our dot blot results, mRNA expression of DNMT1 was higher (1.5 folds) in Q group in both adipose and kidney tissues while no significant differences were observed in liver and muscle. Immunoblot against porcine DNMT1 antibody indicated higher expression of DNMT1 in pigs fed quercetin.

Conclusion: Our study strongly indicates that quercetin might be an inducer for DNA methylation especially in adipose tissue and kidney paving the way to extensively search as to which genes are being methylated thereby shutting down their expressions and how this scenario affects the physiological processes in pigs.

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59.

Effects of an oral supplementation of green tea and curcuma extract on lipopolysaccharide-induced inflammation in horses and ponies

Effekte einer oralen Supplementierung eines Grüntee- und Kurkuma-Extraktes auf den Lipopolysaccharid-induzierten Entzündungsstatus bei Pferden und Ponys

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Introduction: In horses and ponies numerous medical conditions are known to be linked with a status of inflammation in different tissues, especially in the liver. Inflammation can be induced by endoplasmic reticulum (ER) stress, leading to the unfolded protein response (UPR) which can be measured by several markers of the signalling cascade. The influence of feeding a polyphenol-rich supplement consisting of green tea and curcuma extract (GCE) on ER stress and inflammation has been already described in several species such as cattle [1]. Studies in the equine are still lacking. Therefore, the aim of the study was to investigate the expression of selected markers of inflammation in blood and genes involved in inflammation and the UPR in liver tissue after lipopolysaccharide (LPS) challenge with or without feeding a GCE. It was hypothesised that GCE supplementation will reduce inflammatory reactions compared to the placebo. **Animals, material and methods:** A randomized, placebo controlled cross-over study with a wash-out period of 12 weeks was conducted (approved as TVV 34/16). 5 healthy adult warmblood horses and 6 healthy adult Shetland ponies were fed with hay and supplemented for 21 days with a daily dose of 10 g of a blend of green tea catechins and curcumin (20% total polyphenol content) according to manufacturer´s instructions or a placebo (calcium carbonate). After supplementation, all animals underwent an intravenous lipopolysaccharide (LPS) (10 ng/kg BW) challenge with an intravenous infusion over 30 minutes to induce a moderate systemic inflammation. 24 hours before and 12 hours after LPS challenge, blood samples were collected and analysed for serum amyloid A (SAA), haptoglobin and retinol-binding protein 4. In addition, liver tissue was taken transcutaneously under ultrasound control. RT-qPCR was used to analyse liver mRNA expression of selected markers of inflammation and the UPR (*haptoglobin, TNF-α, IL-1β, IL-6, CD68, fibroblast growth factor 21, NF-kB, activating transcription factor 4)*. Liver tissues were histologically examined for inflammatory responses. A commercial software package was used for statistics (STATISTICA). **Results and discussion:** The LPS challenge induced a significant increase in SAA concentration (all values before LPS ≤ 2.6 µg/mL; placebo after LPS: 98.4 113.8 µg/mL; GCE after LPS: 70.7 43.9 µg/mL) reflecting the suitability of LPS to induce systemic inflammation. Hepatic mRNA levels for IL-1β were significantly lower after LPS challenge in supplementation group compared to placebo. Significant differences in other liver parameters were not found. Our findings suggest that feeding a polyphenol-rich supplement offers potential to reduce inflammatory responses in horses and ponies. However, an overall anti-inflammatory effect by a polyphenol supplementation could not be shown in this study and therefore remains open. **Conclusion:** The bioavailability of plant-derived polyphenols is supposed to be low [2]. Therefore target tissues such as liver are probably not affected by the selected dosage of GCE. Low bioavailability might also explain the discrepancies between in vitro and in vivo studies using plant-derived polyphenols as active substances to modify ER-stress and inflammation.

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This study was supported by Kaesler Animal Nutrition, Cuxhaven, Germany.

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60.

Oxidative status and serum concentrations of the acute phase protein haptoglobin in piglets fed either conventional soy bean meal (SBM) or enzymatically treated SBM

Oxidativer Status und Serumkonzentrationen des Akut-Phase-Proteins Haptoglobin bei mit enzymatisch behandeltem Sojaschrot oder konventionellem Sojaschrot gefütterten Ferkeln

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Soy bean meal is an important protein source but contains anti-nutritional substances limiting its use particularly in young animals. Advanced processing procedures such as enzymatic treatment can decrease anti-nutritional factors in SBM. Weaning is known to result in oxidative stress and increased blood concentrations of acute phase proteins (1). We hypothesized that feeding enzymatically treated SBM (ESBM) to weaned piglets would reduce oxidative stress and inflammatory processes when compared to diets containing untreated SBM. **Methods:** 128 weaned piglets (Danbred x Pietrain, average weight 7.5 kg, kept in 32 pens) were fed either a commercial SBM-based diet or a diet containing 136 g/kg of ESBM (HP300, Hamlet Protein, Horsens, DK, patented enzymatic treatment using a proprietary blend of enzymes) instead of SBM from day 1-21 after weaning (phase 1). Thereafter all pigs were fed a commercial SBM based diet (phase 2, day 22-42). Diets were balanced for amino acids and energy. Feed and water were available *ad libitum*. Average daily gain and feed intake were determined per pen at day 7, 14, 21 and 42. Blood samples were taken on day 21 from 10 piglets per treatment from the *V. jugularis externa* and analyzed for haptoglobin (Hp) via ELISA (2), dROM (Derivatives of Reactive Oxygen Metabolites), TBARS (Thio-Barbituric Acid-Reactive Substances), AOPP (Advanced Oxidized Protein Products) and alpha-tocopherol. Analysis of variance (ANOVA) was performed using IBM SPSS Statistics for Windows (Version 21.0). **Results:** Feeding ESBM resulted in a trend (P=0.08) for improved average daily gain in phase 2 compared to SBM (542 g vs. 505 g). Daily weight gain during the whole trial period was not different between the groups (376 g in ESBM and 358 g in SBM, P=0.28). Oxidative status of the piglets was not different between the groups, except for AOPP for which lesser concentrations were observed in the ESBM group (62.3 mg/L vs. 85.5 mg/L, P=0.012). Similarly, the acute phase protein Hp, a marker for inflammatory status, was lower in ESBM than in SBM fed piglets (1.00 mg/L vs. 1.74 mg/L, P=0.028). **Conclusion:** The lower concentrations of Hp and AOPP in ESBM-fed versus SBM-fed piglets indicate that using ESBM may reduce proinflammatory and protein oxidizing reactions. In view of the competition between growth and immune defense for energy and amino acids (3), ESBM-fed piglets may thus have more nutrients available for growth.

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61.

Adipose tissue – do inflammation parameters change within progressive body weight gain in ponies and horses?

Fettgewebe – verändern sich Entzündungsparameter mit Gewichtszunahme in Ponys und Pferden?

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Human obesity goes along with a chronic state of inflammation which is mainly caused by adipose tissue (AT) inflammation. In horses, it is still controversially discussed if equine body weight (BW) gain shows the same effects. The main production site of inflammatory cytokines is the abdominal (abd) AT in humans. On the contrary, in equines the subcutaneous neck AT (neck AT) seems to have a greater contribution to the inflammation. The aim of this study was to evaluate changes of inflammatory parameters in AT depots with BW gain.

Methods: 19 lean and metabolic healthy equines (10 Shetland ponies, 9 Warmblood horses) were were housed in box stalls with access to a sand paddock for 5 hrs/d. Two years equines received 200% of their maintenance energy requirements (metabolizable energy MJ/kg$^{0.75}$; energy covered by hay (60 %) and compound feed (40 %)). Energy intake was adjusted monthly to increased BW. At three sampling points (t0=basal; t1=after one; t2=after two years of hypercaloric diet) AT was sampled in general anaesthesia. Sampled AT depots: abd AT (mesocolon descendens); neck AT. mRNA levels of CD68, IL-1β, IL-6 and TNFα and reference genes (18S and RPL32) were analysed with RT-qPCR. Data are expressed as median (25./75.‰). For time effects data were analysed using Friedmann ANOVA with Wilcoxon test and Bonferroni correction as post hoc test. Different depots were tested with Kruskal-Wallis-ANOVA. Breed effects were evaluated with Man-Whitney-U test. The project was funded by the German research foundation (VE 225/9-1).

Results: Mean BW ± SD increased in ponies and horses with hypercaloric diet. BCS increased in ponies (t0: 2.32 (1.15/3.4); t2: 3.94 (3.7/4.15)) and in horses (t0: 2.65 (2.05/3.2); t2: 3.75 (3.65/3.85)) with BW gain. In horses, CD68 mRNA levels in abd AT were significant higher at t1 ($P = 0.023$) and t2 ($P = 0.023$) compared to t0. In contrast, horses had a decrease in mRNA levels of IL-1β ($P = 0.023$), IL-6 ($P = 0.023$) and TNFα ($P = 0.023$) from t1 to t2 in neck AT. In comparison of the different AT depots horses had higher mRNA levels of CD68 in abd AT compared to neck AT at t0 ($P = 0.001$) and t2 ($P = 0.023$). No significant differences were found concerning CD68, IL-1β, IL-6 and TNFα mRNA levels in ponies with BW gain. But mRNA levels of IL-1β ($P = 0.009$) and IL-6 ($P = 0.003$) were higher in neck AT compared to abd AT in ponies at t2. Comparing the breeds horses had higher CD68 ($P = 0.044$) and lower IL-1β ($P = 0.026$), IL-6 ($P = 0.034$) and TNFα ($P = 0.02$) mRNA levels in neck AT at t0 compared to ponies. At t2 IL-1β mRNA level were higher in abd AT of horses compared to ponies ($P = 0.006$).

Conclusion: An increasing macrophage invasion of abd AT with BW gain was shown by higher mRNA levels of CD68 at t2 in horses compared to t0. On the contrary mRNA levels of IL-1β, IL-6 and TNFα decreased with BW gain in neck AT of horses. The higher CD68 levels in abd AT compared to neck AT might be related to a greater contribution of abd AT to systemic inflammation compared to neck AT. But mRNA levels of IL-1β, IL-6 and TNFα were lower in abd AT compared to neck AT. Interestingly, horses showed lower inflammatory cytokine expression at t0 in neck AT but had higher IL-1β mRNA levels in abd AT at t2. Since no comparison to protein levels could be made the clinical relevance has still to be evaluated. In conclusion the macrophage invasion of AT seemed to increase with BW gain. However, infiltrating macrophages may not promote inflammation because proinflammatory markers decreased in AT with BW gain in horses. In ponies the degree of BW gain may have been to low to induce significant changes in mRNA levels of inflammatory cytokines. The higher mRNA levels of IL-1β and IL-6 in neck AT seemed to confirm that neck AT depot produces more inflammatory cytokines compared to abd AT and the pony differs therefore from humans. Overall at this stage of obesity no increase in inflammatory cytokines could be found in AT of ponies and horses.

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62.

Correlation of gene expression of fibroblast growth factor 21 in the liver of dairy cows in the transition period with genes of stress signaling pathways, inflammation, β-oxidation, and lipogenesis

Korrelationen zwischen der Genexpression von Fibroblast Wachstumsfaktor 21 und Genen von Stresssignalwegen, der Entzündung, der β-Oxidation und der Lipogenese in der Leber von Milchkühen

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Fibroblast growth factor 21 (FGF21) has been identified as a stress hormone which plays an important role in the adaptive response to various stimuli (i.e. nutritional stress, environmental stress) by triggering metabolic adaptations which aim to supply the body with energy, such as a stimulation of lipolysis, ketogenesis, gluconeogenesis or fatty acid oxidation. Recent studies in dairy cows have shown that the expression of FGF21 in the liver is dramatically increased during the transition period. An induction of FGF21 might be helpful to adapt to stress during that time. However, metabolic stimuli of FGF21 induction in dairy cows are yet unknown. The present study aimed to investigate potential metabolic stimuli of the induction of FGF21 in the liver of dairy cows during the transition period.

Methods: 50 Holstein cows (19 primi- and 31 multiparous) were included in the animal experiment, which was conducted at the at the Educational and Research Centre for Animal Husbandry Hofgut Neumühle in Rhineland-Palatinate (Münchweiler an der Alsenz, Germany). The animals were fed a diet which met the requirements of the German Society of Nutrition Physiology. Milk yield, feed intake and body weight were recorded. Blood and liver samples were taken at the time points -2, +1, +4, +7 wks relative to calving. Blood was analyzed for non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) using commercial kits. Plasma Retinol concentration was detected by HPLC. Relative mRNA concentrations of FGF21 and genes related to ER-stress, inflammation, β-oxidation, and lipogenesis were measured by qPCR. Statistical analyses were carried out with the software R (version 3.4.4, R Foundation for Statistical Computing, Vienna, Austria). Pearson's correlation coefficients between various parameters were calculated. For the cluster analysis, the 10 highest or lowest animals in milk yield, negative energy balance (NEB), NEFA, BHBA and FGF21 concentrations were selected. Ls means of the high vs. low group were compared by t-test. Differences in means were considered significant at $P < 0.05$.

Results: Relative mRNA concentrations of FGF21 in the liver were strongly increasing from -2 wk to +1 wk (13-fold in primiparous, 24-fold in multiparous cows). On wks + 4 and +7 relative mRNA concentrations of FGF21 were 10.3./8.3-fold and 4.1/4.1-fold higher, respectively, in primiparous/multiparous cows than on wk -2. Cows with high or low milk yield in average from wk 1 to wk 14 (46 ± 3 vs. 28 ± 4 kg/day, n = 10), high or low NEB (-86 ± 17 vs. -34 ± 10 MJ NEL/d in wk 1, n = 10), high or low plasma BHBA concentrations (1519 ± 409 vs. 401 ± 70 µmol/L, n = 10), high or low NEFA concentrations (872 ± 209 vs. 185 ± 49 µmol/L, n = 10) did not differ in hepatic mRNA concentration of FGF21 on wk +1. On wk +1, there were significant positive correlations between hepatic mRNA concentration of FGF21 and mRNA concentrations of endoplasmic reticulum (ER) stress genes (ATF4, HSPA5), and mRNA concentrations of genes involved in inflammation (ceruloplasmin, haptoglobin (HP)). There were moreover negative correlations between hepatic mRNA concentrations of FGF21 and mRNA concentrations of retinol-binding protein 4 and transthyretin as well as plasma retinol ($P < 0.05$). Cows with high mRNA concentrations of FGF21 in the liver (0,390 ± 0,191) had higher relative mRNA concentrations of genes of ER stress (ATF4, DDIT3, HSPA5, spliced XBP1) and inflammation (HP), and a lower plasma retinol concentration than cows with low hepatic mRNA concentrations of FGF21 (0,009 ± 0,006). Relative mRNA concentrations of lipid metabolism (β-oxidation, lipogenesis) did not correlate with relative mRNA concentration of FGF21.

Conclusion: The data of this study suggest that FGF21 induction in the liver of dairy cows might be induced by ER stress and inflammation. The data moreover shows that milk yield, energy balance, and concentrations of NEFA and BHBA are unrelated to hepatic FGF21 expression.

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63.

Changes in NO production of HD11 chicken macrophages *in vitro* incubated with Masson Pine pollen (*Pinus massoniana*) or *LPS* in the presence and without polymyxin B

Veränderungen in der NO Produktion von HD11-Makrophagen aus Hühnern in vitro bei Inkubation mit Pollen der Massonpinie (Pinus massoniana) oder LPS in Gegenwart und ohne Polymyxin B

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Introduction: Pollen of the Masson Pine (*Pinus massoniana*) have been shown to express positive effects on gut health of pigs (1). Recently, we were able to demonstrate that Masson Pine pollen (MP) activated HD11 chicken macrophages *in vitro* by stimulating TLR4 dependent signalling. In fact, no significant differences were recognized between the cellular response to MP compared to LPS (2). In this follow-up study, we further investigated the effect of MP on NO-production of HD11 chicken macrophages *in vitro* by co-incubation with polymyxin B, an antibiotic that is blocking the lipid A domain of LPS. Thereby, we aimed to further rule out bias by bacterial contamination and to gain more information on the nature of the MP effect on HD11 chicken macrophages.

Methods: 12 MP samples from different production sites and harvest years as well as a certified LPS standard material (E. coli O111:B4, Invivogen) were used. HD11 chicken macrophages were cultivated in a RPMI media containing 1% Glutamax, 8% fetal calf serum, 2% chicken serum and 1% penicillin+streptomycin (Thermo Scientific). Treatment solutions were prepared, containing RPMI media and either 1 mg MP/mL or 1 mg LPS/mL in the presence or without 300 µg of Polymyxin B. All solutions, including untreated RPMI media as negative control, were incubated for 3h shaking. Subsequently solutions were filtered (0.45 µm) and subjected to 0.5fold dilution steps with RPMI yielding a MP/LPS concentration gradient of 0.5, 0.25, 0.125, 0.062, 0.031, 0.0156, 0.0078 0.0039 mg/mL. Cells were incubated in triplicates with one of each treatment solutions for 24h (37°C). Subsequently, NO-production was quantified by applying the Griess Reaction (3). Data was analysed with SAS 9.4 (SAS Institute Inc.) (n = 12 per treatment). Linear regression was applied (Proc Reg) and the effective dosage (ED50) at which 50% of the max NO-production was recognized has been estimated (Proc Probit) and analysed using the T-Test (Proc TTest).

Results: Each treatment solution induced a significant straight linear decrease in NO production in response to the titration gradient ($p < 0.0001$). Furthermore, comparing MP and LPS treated cells with their respective polymyxin B counterparts highlighted a significantly lower NO production ($p < 0.0001$), respectively. The aforementioned linear decrease over polymyxin B treated cells was in each case perfectly parallel to the non-treated MP or LPS counterpart. Estimating ED50 values for single MP samples compared to polymyxin B treated controls yielded significant ($p < 0.0001$) differences in average values of 0.016 compared to 0.029 mg/mL for pure MP and MP+polymyxin B, respectively. In case of LPS, the difference was also significant but the average numbers were in a different range (0.003 compared to 0.04 mg/mL for pure LPS compared to LPS+polymyxin B). Furthermore, comparing ED50 values of pure MP with pure LPS as well as MP+polymyxin B with LPS+polymyxin B showed also a significant differences ($p < 0.0001$) with the LPS treated cells expressing the lowes ED50 values compared to their respective MP counterparts.

Conclusion: HD11 chicken macrophages responded to treatment with MP and LPS respectively. Interestingly, both treatments were less effective in the presence of polymyxin B. However, the effect of the lipid A blocker was more pronounced for LPS compared to MP. In anticipation of data highlighting a minor abundance of microorganisms on the MP samples used in the present study (data not shown), we conclude that MP are interacting with macrophages, presumably through toll-like-receptors in the plasma membrane (2), by a yet unknown pollen-associated molecular motif.

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64.

Analysis of the inflammasome signaling pathway in a porcine intestinal co-culture model exposed to probiotic *E. faecium* and enterotoxigenic *E. coli*

Untersuchung des Inflammasom-Signalweges in einem porzinen intestinalen Kokultur-Modell nach Inkubation mit probiotischen E. faecium und enterotoxischen E. coli

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The intestinal epithelium forms an essential link between the digesta containing a multitude of foreign antigens derived from dietary components and microorganisms, and the underlying mucosa-associated immune cells (1). Among other immune cell populations, dendritic cells (DC) are of enormous importance to regulate downstream inflammatory processes, thereby interacting with intestinal epithelial cells (IEC) (2). The control of invading bacteria involves the recognition *via* innate immune receptors, such as inflammasome-forming nucleotide oligomerization domain-like receptors (NLR). The inflammasome pathway emerged as critical for the host defense against enteropathogenic agents (3). During the post-weaning period of piglets, infections with enterotoxigenic *Escherichia coli* (ETEC) are linked with high losses. Feeding probiotic *Enterococcus* (*E.*) *faecium* constitutes a promising strategy to counter post-weaning diarrhea. The aim of the study was to model epithelial cell-immune cell interactions in a co-culture setup. Of special interest were the involvement of the NLRP3 inflammasome in the response to ETEC and the role of NLRP3 in promoting probiotic effects of *E. faecium*.

Methods: The co-culture setup involved porcine IEC (IPEC-J2) and monocyte-derived DC (MoDC). Porcine MoDC were differentiated from freshly isolated $CD14^+$ blood monocytes. For the co-culture experiments ($N = 3$), Transwell inserts with IPEC-J2 monolayers grown on top were transferred into cell culture plates containing MoDC on the bottom surface. IPEC-J2 and MoDC monocultures served as controls to assess the influence of co-culturing on the reaction patterns of the corresponding cell type. Bacterial challenges were conducted by using a probiotic *E. faecium* and a pathogenic ETEC strain. Samples were taken 6 h after bacteria addition. Several inflammasome-related genes (IL-1β, IL-18, NLRP3, and caspase-13, the latter as a candidate for the non-canonical inflammasome pathway) were analyzed by quantitative real-time PCR. At the protein level, IL-1β secretion was detected by ELISA. Data were statistically analyzed by two-way repeated measures ANOVA and the Fisher least significant difference *post hoc* test.

Results: In porcine MoDC, the mRNA expression of IL-1β, IL-18, and NLRP3 increased after ETEC exposure ($P \leq 0.05$). With regard to IL-1β and NLRP3, the upregulations were significantly higher in MoDC monocultures compared with co-cultured MoDC ($P \leq 0.05$). This was, as a trend, also verifiable for IL-18 mRNA and IL-1β protein expression by MoDC. The mRNA levels of caspase-13 were equally enhanced by ETEC both under mono- and co-culture conditions in MoDC ($P \leq 0.05$). In IPEC-J2 cells, an upregulation of caspase-13 expression due to ETEC treatment was detected ($P \leq 0.05$), which was greater under co-culture conditions ($P \leq 0.05$). Beyond that, NLRP3 mRNA expression in IPEC-J2 cells increased upon ETEC stimulation ($P \leq 0.05$).

Conclusion: The results indicate that ETEC, in contrast to *E. faecium*, affects the inflammasome pathway in IPEC-J2 cells and porcine MoDC. Modulation of porcine caspase-13 by infection with ETEC occurred in both cell types. In the presence of IPEC-J2 cells, MoDC show a more tolerogenic phenotype. To elucidate this regulation further will be the target of future studies.

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65.

Changes in the microbiome of caecal content and excreta in broilers induced by a reduced dietary protein content

Mikrobiomveränderungen in Caecuminhalt und Exkrementen von Broilern nach proteinreduzierter Fütterung

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A protein reduced diet (PRD) in broilers could help to decrease total nitrogen in excreta, facilitating the compliance of the fertilizer ordinance. Serine, aspartate, glutamate and proline are essential for *C. jejuni*. These nutrients were reduced to decrease an artificial *C. jejuni* infection. The listed amino acids were given in reduced quantity, while indispensable amino acids were held in a comparable level in both groups (1). Microbiome studies were performed in order to analyze changes induced by a PRD.

Animals, Material and Methods: In total 150 broilers (Ross 308) were divided into 10 groups of 15 animals each. After 7 d rearing and 7 d adaptation period, the experimental phase began with groups fed either control diet (CD; 212g XP, 455g starch, 13.3g Lys /kg DM) or a protein reduced diet (PRD; 190g XP, 499g starch, 12.6g Lys /kg DM) with decreased amounts of soybean meal and essential amino acids given additionally. Three birds each (seeders) were infected orally on d 21 with 1.5 x 10^4 cfu *C. jejuni* per 2ml infection dose. For microbiome studies caecal contents and excreta of the seeder birds (30/150; 15 CD and 15 PRD) were taken on d 23 and on d 32 from excreta and on d 44 from caeca. Samples were stored at -80 C until chyme and excreta were homogenized. DNA-extraction was done on a liquid handling automate; based on the DNeasy Blood&Tissue Kit. Amplification of the 16S rRNA gene was done on hypervariable region V 4. The amplicons were sequenced on the Illumina-Miseq platform. DNA reads processing and statistical analysis was performed using QIIME (version 1.8.0) and MicrobiomeAnalyst.

Results and Discussion: Birds stayed clinically healthy in all groups throughout the trial. Feed intake and body weight did not differ significantly until dissection. On d 23 *C. jejuni* (cfu in excreta) were reduced significantly. Feeding PRD was associated with an increase of *Turicibacteraceae* and a decrease of *Clostridiaceae* in excreta on d 23. In addition, the number of *Enterobacteriaceae* was in tendency lowered in the PRD group. Taking both diets into account a decrease of the dominating family of *Lactobacillaceae* (68.6 % to 44.4 %; $p < 0.05$) between d 23 and d 32 and an increase of *Clostridiaceae* (7.07 % to 18.6 %; $p < 0.05$) and *Turicibacteraceae* (1.14 % to 8.55 %; $p < 0.05$) in excreta of *C. jejuni* infected broilers were detected. On d 23 and d 32 significantly more *Turicibacteraceae* ($p = 0.03$) were seen with PRD. Lower amounts of *Clostridiaceae* were observed with PRD at d 23 and 32, but the latter could not be statistically confirmed. In caecal contents on d 44 most abundant order was still *Clostridiales*, but on the family level most abundant was *Ruminicoccaceae* while *Clostridiaceae* were only a minor group. With PRD only minor groups (Coriobacteriaceae and Blautia) decreased significantly on d 44 while *Turicibacteraceae* and *Clostridiaceae* increased in tendency. Higher numbers of *Clostridiaceae* in chickens fed higher protein were found in different studies (2). The role of *Turicibacter* sp. in chickens is not clear by now, but was found with higher amounts of starch (3) and with age (3).

Conclusion: The intake of PRD altered the fecal microbiome in broilers significantly, whereas no impact on clinical health status was observed. In excreta increased amounts of *Turicibacteraceae* at both timepoints and a decrease of *Clostridiaceae* at d 23 were observed. Eventually, less differences between the two feeding groups were seen with increasing age.

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66.

Effects of supplementation of methionine on growth performance and antioxidant status of broilers under condition of heat stress

Effekte einer Methioninsupplementierung auf das Wachstum und den antioxidativen Status von Broilern unter Hitzestress

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It is well known that exposure of broilers to high ambient temperature causes heat stress which in turn impairs growth performance and causes oxidative stress. Methionine (Met) is known to play a role in the antioxidant system of the body as it acts as the pre-cursor of the synthesis of cysteine which is required for the formation of glutathione (GSH), one of the major antioxidants in animal tissues. The present study aimed to investigate the hypothesis that supplementation of the diet with Met in excess of the requirement for optimum growth performance alleviates the adverse effects of heat stress on growth performance and the antioxidant status in broilers. Moreover, it was intended to elucidate whether two commercial sources of Met, DL-methionine (DLM) or DL-2-hydroxy-4-(methylthio) butyric acid (DL-HMTBA) have different effects in this respect.

Methods: Male Cobb-500 broilers (n=102) were allotted to six groups and phase fed three wheat-soya bean meal based basal diets during days 1-10, 11-21 and 22-35. The Met + cysteine (Cys) concentrations were about 10% lower than those recommended by the breeder and about 20% lower than those recommended by Evonik (AMINODat® 5.0). One group was kept under thermoneutral condition and received the basal diets. The other five groups were kept in a room with an increased ambient temperature [27.4 ± 0.3 (SD) ºC] from weeks 3-5, and were fed the basal diet or the basal diets supplemented with two levels of either DLM or DL-HTMBA on an equimolar base (0.19 and 0.37% in starter diets, 0.16 and 0.32% in grower diets, 0.15 and 0.29% in finisher diets), yielding Met + Cys concentrations which were around 10% and 25-30%, resp., in excess of the recommendations of the breeder. Concentrations of antioxidants (vitamin C, tocopherols, GSH) were measured by HPLC, concentrations of thiobarbituric acid-reactive substances (TBARS) by a floures-cence photometer and relative mRNA abundances of stress responsive genes by qPCR. Data were analysed by two-way ANOVA.

Results: Heat exposed broilers fed the control diets showed a lower feed intake and lower body weight gains than broilers kept under thermoneutral conditions ($P<0.05$). They also had a higher respiration rate (breaths/min), a lower tocopherol concentration in plasma and liver, a lower concentration of vitamin C in the liver, and a higher ratio between reduced and oxidized glutathione (GSH:GSSG) ($P<0.05$). Concentrations of TBARS in liver, plasma and muscle did not differ between both groups, however heat exposed broilers had a higher relative mRNA concentration of antioxidant genes (catalase) and genes of ER stress [activating transcription factor (ATF) 4, ATF6] than broilers kept under thermoneutral condition ($P <0.05$). Supplementation of heat exposed broilers with DLM or DL-HTMBA did not improve animal performance (feed intake, body weight gain, feed:gain ratio) or respiration rate but led to an increase of GSH concentration in liver and breast muscle and to a reduction of the TBARS concentration in the liver ($P<0.05$). However, concentrations of tocopherols and vitamin C as well as relative mRNA concentrations of stress responsive genes in the liver remained unchanged by Met supplementation.

Conclusion: The study confirms that heat exposure impairs growth performance and causes oxidative stress in broilers. The finding that Met supplementation in heat stressed broilers did not improve growth performance indicates that the control diet was already sufficient for Met + Cys. It is shown that supplementation of Met enhances the formation of GSH in liver and muscle of heat exposed broilers. However, as concentrations of vitamin C, tocopherols and gene expression of stress responsive genes were not influenced, it is concluded that Met supplementation had overall only moderate effects on the antioxidant system in heat exposed broilers. Moreover, it was found that there was no difference in the effects observed by the two Met sources used, DLM and DL-HTMBA.

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67.

Intensively expander processed corn in broiler diets: impact on apparent ileal amino acid digestibility at different stages of fattening

Intensive Expanderbehandlung von Mais: Einfluss auf die scheinbare ileale Verdaulichkeit von Aminosäuren bei Broilern unterschiedlichen Alters

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The recommendations for energy and nutrient requirements of broiler chickens (1) assume that amino acid (AA) digestibility of raw feed materials, like corn, do not depend on age. However, different studies have reported an increased pancreatic enzyme concentration during the early posthatch period of broiler chickens. Furthermore, intensive hydrothermic processing is known to result in chemical and physical modifications, which might improve feed digestibility in young broiler chickens. In contrast, intensive processing may promote Maillard reaction, impairing AA utilization. This study aimed to investigate if intensively expander processed corn increase the apparent ileal digestibility (AID) of AA in dependence on birds' age.

Methods: 288 one-day-old broiler chickens (Ross 308) were randomly allotted to one of three different dietary treatment groups (16 animals per pen, 6 replicates per treatment). The control group (C) received diets containing conventionally dried corn, which was quantitatively replaced by short (SC, 60 s) or long term pre-steam-conditioned (LC, 1080 s) and intensively expander processed (approx. 45 kWh/t SME (specific mechanic energy input, kWh/t) using expander Model OEK 15, Amandus-Kahl, Reinbek, Germany) corn of the same batch. Corn treatments were included at 519 g kg^{-1} in the grower diet (d 9-22) and 574 g kg^{-1} in the finisher diet (d 23-35). Processed or unprocessed corn, respectively, was mixed with further components (soybean meal, grass meal, feed fat and premix) to meet or exceed recommended levels (2). For determination of AID of AA, TiO_2 as an inert marker was used throughout the trial (3.0 g kg^{-1}). All animals had *ad libitum* access to feed and water. At the end of the grower and finisher phase (d 22 or d 35 of fattening), four representative broiler chickens of each pen were selected and slaughtered for collection of ileal digesta which was pooled. Analysis of AA (except tryptophan and tyrosine) of corn, diets and ileal digesta was conducted (3) by ion exchange chromatography on the Biochrom 30 system (Biochrom Ltd., Cambridge, UK). Furthermore the KOH-protein solubility of hydrothermic processed corn was analysed. Data were analysed using ANOVA (treatment, age, treatment x age) assuming a randomized block design, with Tukey-Kramer test for LS-mean separation ($P<0.05$) (SAS 9.4, SAS Institute Inc.).

Results: Most of the AA were not affected while the content of methionine and cysteine was reduced by hydrothermic processing in experimental diets. Furthermore KOH-protein solubility decreased markedly with increasing treatment intensity (C, SC and LC: 87.1%; 80.0% and 70.6%). No interaction was evident between age and hydrothermic treatment regarding AID of AA ($P>0.10$). Broiler chickens of the finisher phase (d 35) compared to grower birds (d 22) expressed increased AID of cysteine (73.5 *vs.* 67.1%; $P<0.05$) and tented to express higher AID of serine (79.8 *vs.* 77.5%; $P<0.10$) irrespective of the dietary treatment. In contrast, grower birds showed higher AID of isoleucine (78.3 *vs.* 75.6%; $P<0.10$). Irrespective of the preconditioning time of corn (SC *vs.* LC) prior to intensive expander processing, hydrothermic treatment decreased AID of most of the essential and nonessential amino acids ($P<0.05$) excluding lysine, cysteine ($P<0.10$), threonine and serine ($P>0.10$).

Conclusions: There was no evidence that intensive expander processing of corn may facilitate utilization of corn-soybean based broiler diets in the early life stage. This may have been due to increased degradation of sulfur-containing amino acids and reduced KOH-protein solubility during intensive hydrothermic processing of corn.

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68.

Protein intake of broiler chickens in choice experiments after experimental infection with *Campylobacter jejuni*

Proteinaufnahme von Broilern in Wahlversuchen nach experimenteller Infektion mit Campylobacter jejuni

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Campylobacteriosis has been the most commonly reported zoonosis in the European Union since 2005. The infection is often linked to the consumption and handling of poultry meat [1]. Unlike most other intestinal bacteria, *Campylobacter* have a limited ability to use carbohydrates for their metabolism and rely on the use of amino acids [2]. The aim of the present study was to investigate whether an infection with *C. jejuni* alters the feed intake of broiler chickens with special regard to the level of protein intake.
Methods: After a fourteen-day rearing phase a total of 300 Ross 308 broiler chicken were randomly divided into four groups with five replications each [20 subgroups (15 animals/subgroup) in a 2 x 2 factorial design with two different diets and a different infection modus (CN, *Campylobacter* Negative, CP, *Campylobacter* Positive)] and kept for further 28 days in boxes littered with wood shavings (1 kg/m^2). Two of the four groups were fed *ad libitum* a standard diet (SD-diet: 216 g XP/kg DM, 14.4 MJ AME_N/kg DM). The other two choice diet (CD-diet) groups were given the opportunity to freely choose between two different compound feeds based on the SD-diet but modified in their content of wheat and soybean meal in order to generate different levels of crude protein (CD^{XP+}-diet: 286 XP/kg DM, 13.9 MJ AME_N/kg DM, and CD^{XP-}-diet: 109 XP/kg DM, 15.1 MJ AME_N/kg DM). An intake of both CD-diets at a ratio of 3:2 (CD^{XP+}-diet: CD^{XP-}-diet) resulted in a composition of consumed feed identical to that of the SD-diet concerning composition, energy and nutrient content. At day 21, in each subgroup of SDCP and CDCP, three of 15 broilers were orally infected with a suspension containing 5.26 $\log_{10}$ cfu of *C. jejuni*. Individual samples in groups SDCP and CDCP and regular spot-checks of five randomly selected animals per subgroup (SDCN and CDCN) were taken and examined for *C. jejuni* occurance. The body weight was recorded individually at day 7, 14, 21 and 42 while the feed and water intake were monitored at subgroup level weekly. The differences in means between the groups were tested by two-factorial analysis of variances with “diet” and “infection” as independent variables as well as multiple pairwise comparisons between combinations of variables (significance level: $p < 0.05$).
Results: Between d 21 to d 42, the SD-diet fed subgroups showed a higher daily feed intake per animal (156 ± 4.50 g) than the subgroups fed the CD-diet (151 ± 3.70 g). The final body weight (d 42) was significantly different between the groups fed SD-diet (SDCN: 3373 ± 369 g, SDCP: 3307 ± 391 g) and the experimentally infected groups of the CD-diet (3242 ± 419 g) in comparison to non-infected groups fed CD-diet (3037 ± 397 g). Nevertheless, the body weight of all groups at d 42 was higher than the target of 2809 g [3]. Seven days after experimental infection 100% of animals in the experimentally infected groups were *C. jejuni* positive. A nearly identical feeding behavior was observed between the groups with choice diets in the period before the experimental infection (d14-20, CDCN: 14.4 g XP/animal and CDCP: 15.2 g XP/animal). Over the first two weeks after the experimental infection (d 21-27 and 28-34), the protein intake in the experimentally infected subgroups offered CD-diet was significantly higher (26.3 and 33.2 g/animal, respectively) than in the CD-diet fed subgroups without infection (24.8 and 30.9 g/animal, respectively). This resulted in a significantly higher content of crude protein in the consumed diet (198 ± 3.09 g XP/kg DM and 208 ± 8.57 g XP/kg DM, respectively).
Conclusions: Broiler chickens alter their feed intake behaviour in terms of protein intake under the infection with *C. jejuni*. Therefore, targeted changes of crude protein levels in compound feed for broilers may affect the infection dynamics of *C. jejuni* and could contribute to develop effective feeding strategies in order to limit the spread of *C. jejuni* infection in cickens.

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69.

Application of an *in vitro* multi-enzyme assay to estimate the precaecal digestibility of crude protein and amino acids in broiler chicken

Anwendung einer in vitro Multienzymmethode zur Schätzung der praecaecalen Rohprotein- und Aminosäurenverdaulichkeit bei Broilern

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Knowledge of the precaecal (pc) crude protein (CP) and amino acid (AA) digestibility is used in formulation of poultry diets. The pc CP and AA digestibility can differ between various feedstuffs as well as between batches of the same feedstuff. Thus, table values can provide misleading information. However, they are generally used because there is no standard method to predict the actual pc CP and AA digestibility. Thus, the aim of the study was to evaluate the use of an *in vitro* multi-enzyme assay to predict the pc digestibility of CP and AA in broiler chicken.

Methods: For this purpose, the *in vitro* rate of CP and AA disappearance of different single feedstuffs (wheat, rye, triticale, spring barley, buckwheat, millet, naked barley, naked oats, spelt, field pea, field bean, lentil, alfalfa leafs, two grass-clover-silages, and maize kernel silage) was related to the pc CP and AA digestibility in 21- and 42-day old broiler chicken (1). In all cases CP analysis was conducted according to Dumas (N*6.25) and AA analysis was conducted using HPLC.

A modified *in vitro* multi-enzyme assay with pepsin and pancreatin (1, 2) was applied to simulate the digestion in broiler chicken and, consequently, to determine the *in vitro* rate of CP and AA disappearance. The rate of the CP and AA disappearance was calculated as the difference between the CP and AA contents in the feed samples and the residues.

The pc CP and AA digestibility of the single feedstuffs was determined in 21- (d21, in 16 feedstuffs) and 42-day old (d42, in 14 feedstuffs) broiler chickens using a linear regression approach (3). Diets containing three different levels of the test feedstuff in exchange for maize starch and a defined amount of the marker TiO_2 were fed to birds for one week until they reached either 21 or 42 days of age. The test feedstuff was the sole source of additional CP and AA. The birds were killed by asphyxiation with CO_2 and the digesta was gently flushed from the terminal two thirds of the ileal section between Meckel's diverticulum and 2 cm prior to the ileo-colonic junction with distilled water. The intake of the AA or CP was related to the precaecally digestible amount of the test AA or CP by linear regression analysis. The slope of the regression is a predictor for the pc digestibility coefficient.

Linear regression analysis was used to relate the *in vitro* rate of CP and AA disappearance to the pc CP and AA digestibility in broiler chicken.

Results: The rate of CP and AA disappearance was strongly related to the pc CP digestibility. The coefficient of determination of regression analysis with eleven data points was high; It amounted to 0.87 for 21-day old broiler chicken and 0.94 for 42-day old broiler chicken. However, due to problems in the separation process of the sample from the fluid, the *in vitro* rate of CP and AA disappearance could not be determined for naked barley. The pc CP and AA digestibility of alfalfa leafs was not in accordance with the *in vitro* rate of disappearance. Due to unsatisfying *in vivo* results (coefficient of determination of the slope <0.7), the data for winter rye (d21 and d42), spelt (d21), and naked oats (d21) was also deleted from the regression analysis.

Conclusions: The adapted *in vitro* multi-enzyme assay provides good estimates for the pc CP and AA digestibility of single feedstuffs for broiler chicken. Further calibrations with *in vivo* results are recommended to improve the significance of the assay. Various samples of different feedstuffs as well as of mixed feeds with known pc digestibility of the CP and AA should be used for this purpose.

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The project was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and Other Forms of Sustainable Agriculture (BÖLN 2811OE054).

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70.

Investigations on the optimal arginine supply for performance and carcass parameters in turkeys

Untersuchungen zum optimalen Arginin-Gehalt für Performance und Fleischansatz bei Puten

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Introduction: Environmental concerns and resource-efficient animal production are key drivers of industry to formulate low crude protein (CP) diets. Crystalline amino acids (AA) are inevitably necessary in these diets in order to avoid performance losses. In classical corn-soybean-meal diets L-arginine (Arg) is regarded as 5th limiting amino acid for broilers (Fernandez et al. 1994). Several studies investigated the Arg requirements for meat-type chickens. However, less information is available for turkeys. Aim of this study was to determine the Arg requirements of turkeys for performance and carcass parameters.

Animals, materials and methods: The trial was conducted with 624 BUT 6 hybrid female turkeys, which received a 6-phase feeding program (P1: 1-21 days; P2: 22-42 days; P3: 43-56 days; P4: 57-70 days; P5: 71-84 days; P6: 85-105 days). The birds were fed with one of six treatment diets (calculated values): T1) basal diet without Arg supplementation (P1: 8.7 g/kg dArg); T2) Arg content 33 % below the recommendation (P1: 11.9 g/kg dArg); T3) Arg content 16.7 % below the recommendations (P1: 14.8 g/kg dArg); T4) meeting Arg recommendations (Aviagen Turkeys) (P1: 17.7 g/kg dArg); T5) Arg content 16.7 % above the recommendations (P1: 20.7 g/kg dArg) and T6) Arg content 33 % above the recommendations (P1: 23.5 g/kg dArg). The lysine (Lys) content of all diets was kept stable (P1: 17.3 g/kg dLys; P2: 15.3 g/kg dLys; P3: 13.5 g/kg dLys; P4: 11.9 g/kg dLys; P5: 10.4 g/kg dLys; P6: 9.3 g/kg dLys). Diets were formulated on the basis of corn and soybean meal and were provided *ad libitum.* A part of the animals was slaughtered at day 106 following a 24-hours fasting, in order to measure the carcass parameters (drumstick, breast, back, neck, wings, liver, abdominal fat). Results were analysed by ANOVA. Tukey-test (SAS, 2004) was applied to compare treatment groups at the $p < 0.05$ level.

Results and discussion: The birds fed on the basal diet (T1), had significantly lower weight gain, feed intake, FCR and carcass parameters than birds fed with Arg supplemented diets. Supplementation of Arg to a deficient basal diet improved the turkey's performance and carcass parameters in a dose-response manner. Numerically the highest average daily weight gain in all periods was observed in the group which received T5 (e. g. d 1 - 105: T1: 99.3^{a} g; T5: 103.7^{b} g). The highest breast meat yield was observed in the treatment (T6) with the highest Arg supplementation, even though it seems that an Arg content of 16.7 % above the recommendations is enough to reach prime Results: However, these results couldn´t be statistically secured. The evaluation of results through the regression analysis is still ongoing.

Conclusion: Modern turkeys may benefit from a higher dietary Arg content as compared to Aviagen recommendations. However, further investigations are required to receive more insights into the Arg requirements of turkeys.

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71.

Free amino acid pattern in serum of fattening turkeys suffering from hepatic lipidosis

Gehalte freier Aminosäuren im Blut von Mastputen unter dem Einfluss einer Hepatischen Lipidose

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Hepatic lipidosis (HL) is a well-known disease in fattening as well as in parent turkey flocks. A lot of underlying reasons have been discussed, but the concrete etiology and pathogenesis are still not clarified. Besides questions primarily concerning fat metabolism, dietary effects like low protein diets with low contents of distinct essential amino acids are considered to be a potential predisposition (Popp, Hauck et al. 2014). For example methionine as an intermediate for carnitineand several lipoproteins and its function as a lipotrophic factor should be investigated. Several studies reported abnormal amino acid profiles in hepatic disease of humans and other species.In humans elevated plasma branched-chain amino acids (BCAA) are associated with insulin resistance and the ratio of BCAA to aromatic amino acids (AAA) in plasma is used to predict hepatic necrosis and cirrhosis.

Methods: In cooperation with German poultry veterinarians three cases of hepatic lipidosis (HL) on three different fattening turkey farms (13/14 weeks old, ”B.U.T. 6” and “TP7”) are in the focus of this study. Per case, up to 20 birds with clinical signs were taken and up to 10 clinically non-affected birds (NA) were taken randomly during at least two consecutive days of illness. Overall, 73 birds were examined and blood samples were taken from each bird. Additionally, 15 blood samples of healthy slaughtered animals (SA; 15 weeks, B.U.T. Big 6) were investigated. For the statistical analysis of the data the Statistical Analysis System SAS® Enterprise Guide®, version 7.1 (SAS Institute Inc. Cary, USA) for windows was used. Overall the data were not normally distributed and the Wilcoxon test was carried out.The values are given as mean values and standard deviation.

Results: In general, the total amount of the constituent amino acids, ammonia and urea in blood samples were the highest in the group of animals with HL in the same way like the sum (431^{a}± 110 mg/dl serum) of these compounds, which was more than three times higher than the sum among SA (134^{b}± 11 mg/dl serum) and NA (114^{c} ± 17.2 mg/dl serum).Methionine was highest among animals with HL (5.94^{a}±2.12), lowest among SA (1.06^{c}±0.12) and in between among NA (1.27^{b}±0.35). The amount of BCAAs (isoleucine, leucine and valine) was highest among animals with HL (22.0^{a}± 7.29 mg/dl serum), lowest among NA (9.38^{c}± 2.06 mg/dl serum) and in between among SA (12.9^{b}± 1.12 mg/dl serum).The amount of aromatic amino acids (AAA) (tryptophan, tyrosine and phenylalanine) was also highest in the group of animals suffering from HL (27.0^{a}± 8.18 mg/dl serum) but lowest in the group of SA (5.95^{c}± 0.59 mg/dl serum) and in between in the group of NA (6.79^{b}± 1.30 mg/dl serum).The ratio of BCCA to AAA was with an average of 2.19^{a}± 0.21 highest among SA, lowest among animals with HL with an average of 0.85^{c}± 0.26 and in between among NA with an average of 1.42^{b}± 0.40.

Conclusions: The high level of methionine in blood samples among the animals with HL could suggest an increased mobilization and usage (Ma, He et al. 2018)and a decreased serum ratio of BCAAs to AAAs is a hallmark of liver cirrhosis among human patients with liver disease and decreases with its progression (Kawaguchi, Izumi et al. 2011). A large part of results are in between among NA, which could suggest an early alteration in the metabolism of protein and amino acids.

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72.

Effects of leucine supplementation on growth performance and biochemical pathways of protein synthesis and protein degradation in muscle and liver of broilers

Auswirkungen einer Leucin-Supplementierung auf das Wachstum und die biochemischen Wege der Proteinsynthese und des Proteinabbaus in Muskel und Leber von Broilern

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Studies in rats, pigs and humans have shown that administration of very high doses of leucine (Leu) can activate skeletal muscle protein synthesis and inhibit degradation of protein in skeletal muscle and liver tissue. However, studies on the effects of leucine supplementation in broilers are scarce. The aim of the present study was to investigate the hypothesis that dietary supplementation of high doses of leucine activates protein synthesis and inhibits protein degradation pathways which results in increased breast muscle yield in broilers. Therefore, gene and protein expression of different pathways involved in protein synthesis and degradation were determined in breast muscle and liver of broilers after a 5-week feeding trial.

Methods: A total of 180 day-old male chicks (Cobb500) with an initial body weight of 41.4 ± 3.6 g (mean ± SD) were assigned to 3 groups and fed basal diets consisting mainly of corn, wheat, peas and soybean meal in 3 phases (starter: days 1-10, grower: days 11-21, and finisher days: 22-35). The control group (L0) received the basal diet which met the broiler´s requirements of nutrients and amino acids for maintenance and growth, and contained Leu:Isoleucine (Ile) and Leu:Valine (Val) ratios close to those recommended by the breeder. Groups L1 and L2 received basal diets supplemented with Leu to exceed NRC recommendations by 60 and 90%, respectively, and the other branched-chain amino acids (BCAA) Ile and Val were supplemented to keep Leu:Ile and Leu:Val ratios fixed. Feed intake and body weight were recorded and samples of plasma, liver, breast muscle, and pancreas tissue were collected on days 10, 21 and 35. Amino acids and keto-acids were analysed using a Biochrom 20 amino acid analyser (Biochrom Ltd., Cambridge, U.K.). Pancreatic enzyme activity of the branched-chain α-keto acid dehydrogenase (BCKDH) was determined photometrically. Relative mRNA abundances were determined by qPCR. Protein expressions were detected using Western Blot analyses. All the statistical analysis was done using the Minitab Statistical Software (Rel. 13.0, Minitab Inc., State College, PA). Differences between groups were analyzed using the Tukey test and means were considered significant at $P < 0.05$.

Results: Feed intake, body weight gain, and feed conversion ratio did not differ between the groups ($P > 0.05$). Plasma concentrations of Leu, Ile, Val, their keto acids, and activity of BCKDH in the pancreas increased dose-dependently with increasing BCAA concentrations in the diets. Relative mRNA expressions and abundances of total and phosphorylated proteins involved in the mammalian target of rapamycin (mTOR) pathway of protein synthesis, the ubiquitin-proteasome pathway and autophagy-lysosomal pathway of protein degradation, the GCN2/eIF2a pathway involved in inhibition of protein synthesis, and the myostatin–Smad2/3 pathway involved in myogenesis were mainly unchanged in breast muscles of broilers supplemented with Leu compared to control broilers. The mRNA abundances of genes involved in the growth hormone axis and autophagy-related genes in breast muscles and livers remained also largely unaffected by supplementation of Leu.

Conclusion: Dietary supplementation of Leu in excess of the requirement for maximum growth with constant Leu:Ile and Leu:Val ratios does not influence protein synthesis or degradation pathways in breast muscle, and subsequently does not increase muscle growth in broilers.

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73.

Precaecal amino acid digestibility and $InsP_6$ disappearance in broiler diets containing different oilseed meals as influenced by phytase supplementation

Beeinflussung der praecaecalen Aminosäurenverdaulichkeit und des $InsP_6$-Abbaus durch Phytasezusatz in Broilerrationen mit verschiedenen Extraktionsschroten

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Reports whether precaecal (pc) amino acid (AA) digestibility is increased by phytase supplementation or not are contradictory. The allocation of phytate ($InsP_6$) and storage proteins differs between different oilseeds. This might affect degradation of $InsP_6$-protein complexes by supplemented phytase. The aim of the present study was to investigate whether effects of phytase supplementation on pc $InsP_6$ degradation and pc AA digestibility differ between broiler diets containing different oilseed meals.

Methods: Three diets based on maize and different protein feeds were used. In one diet, soybean meal (SBM) was the sole protein feed. In the two other diets, SBM (250 g/kg) was combined with either rapeseed meal (RSM; 200 g/kg) or with sunflower meal (SFM; 300 g/kg). Diets were mixed with or without 1500 FTU phytase/kg (Natuphos E 5000 G) and fed to a total of 450 broiler chickens (5 pens per treatment, 15 birds per pen). Diets were formulated to meet or exceed the recommendations of the GfE (1) and were therefore supplemented with limestone, monocalcium phosphate, and free amino acids. The birds were kept on wooden shavings and were offered a commercial starter diet until day 16. From day 16 to 21, the animals received the experimental diets and were housed on perforated floor. On day 21, digesta from the terminal half of the section between Meckel's diverticulum and 2 cm anterior to the ileo-ceco-colonic junction was flushed out using ice-cold deionised water and pooled on a pen basis. Samples were analysed for the concentrations of AA, InsP isomers and TiO_2 as indigestible marker. Results were statistically analysed considering the oilseed meal, phytase supplementation and the interaction between these factors as fixed effects, and the pen as a random effect at a significance level of $\alpha = 5\%$ (proc mixed of SAS 9.4).

Results: Regarding pc $InsP_6$ disappearance, the interaction between oilseed meals and phytase supplementation was significant. Without phytase supplementation, $InsP_6$ disappearance differed significantly between the diets (RSM 2 %, SBM 22%, SFM 12 %). Phytase supplementation increased pc $InsP_6$ disappearance by 68 (RSM), 59 (SBM), and 51 percentage points (SFM). However, there was no significant interaction in regard to the amount of $InsP_6$ that was degraded by phytase (12 µmol/kg DM on average of all diets). The pattern of InsP isomers in the ileum digesta was changed by phytase supplementation in a similar way for all oilseed meals. Supplementation of phytase led to a significant shift from $InsP_6$, Ins(1,2,3,4,6)P_5 and Ins(1,2,4,5,6)P_5 towards Ins(1,2,3,4,5)P_5 and $InsP_4$ isomers, and myo-inositol. The mean increase of pc digestibility by phytase supplementation across all AA was 0.8 percentage points for SBM and 1.4 percentage points for RSM and SBM. Except for Met, pc digestibility of all AA and CP was significantly increased by phytase supplementation. A significant interaction between phytase supplementation and the added oilseed meal on pc digestibility was not detected for any AA. With exception of Cys and Trp, the increase in pc digestibility was numerically lower for SBM than for RSM and SFM.

Conclusions: Results indicate that supplemented phytase can hydrolyse the same amount of $InsP_6$ when different oilseed meals are used in the diet. Because $InsP_6$ concentration is different between oilseed meals, the dosage of phytase needed to achieve the potential of $InsP_6$ degradation may need to be adjusted to the main protein source used in the diet. Results also indicated by a trend that phytase effects on pc AA digestibility differ between oilseed meals and are higher when RSM or SFM are used compared to SBM.

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74.

Effects of hydrolysing the protein sources (wheat gluten/rice protein) on apparent digestibility of nutrients in dogs

Auswirkungen einer Hydrolyse der Proteinquellen (Weizengluten/Reisprotein) auf die scheinbare Verdaulichkeit der Rohnährstoffe bei Hunden

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Introduction: Food sensitivity is one of the most common causes of non-seasonal allergic skin diseases in dogs that are associated with the ingestion of a substance present in the diet [1]. One strategy for reducing allergenicity involves hydrolysing the protein source with enzymes [1]. By enzymatically breaking the protein down into smaller peptide fragments it becomes intrinsically less allergenic and more digestible. When the protein is properly degraded before contact with the gastrointestinal mucosa, it will not activate the immune system [1]. Diets based on hydrolysed protein sources represent the ´next generation´ of commercially available elimination diets [2]. The object of this study was to compare diets with protein sources that had or had not been hydrolysed prior to extrusion regarding the apparent nutrient digestibility and parameters of faeces composition.

Methods: In a cross over model 6 intact adult female beagles were fed 2 different diets: Both were produced under the same extrusion technique and differed only in the pretreatment of the protein sources, depending on whether they had or had not been hydrolysed before. Due to the hydrolysis the solubility of amino acids in water (dilution 1:40) increased significantly, but in different degrees (e. g.: leucine wheat gluten 1.27%/ rice protein 7.96%). The dogs were fed according to their energy requirements. For testing the digestibility faeces were collected for 5 days after an adaptation period of 5 days. The faeces´ consistency was scored (1=very hard; 2=solid, well formed; 3=soft, still formed; 4=poor consistence; 5=watery diarrhea) and other faecal parameters like mass and DM content were determined. Statistical analyses were done using the SAS® software.

Results: The nutrient content of both experimental diets were considerable alike (nutrient content (g/kg DM): not hydrolysed OM 945, XP 222, XL 106, NfE 600; hydrolysed OM 942, XP 256, XL 106, NfE 562). The faecal score and the defecation frequency per day were quite similar in dogs fed both diets (score: not hydrolysed 2.10±0.088 hydrolysed 2.18±0.203; defecation frequency/day: 2.33±0.206, 2.47±0.393). The DM content was significantly different ($p<0.05$) between the different dietary treatments (DM content (%): not hydrolysed 29.0^{a}±0.969 hydrolysed 27.6^{b}±1.53). The apparent digestibility rates (aD) for the whole diet were quite similar as well, except for the apparent digestibility of crude protein (aD [%]: not hydrolysed OM 85.5±0.724, XP 78.5^{a}±2.99, XL 94.0±0.307, NfE 89.5±0.783; hydrolysed OM 85.2±0.894, XP 81.1^{b}±1.98, XL 93.4±0.982, NfE 88.9±1.35). The apparent digestibility of crude protein was significantly ($p<0.05$) improved by pretreatement (hydrolysing).

Conclusions: The allergic reaction cannot be tested because the experimental animals were healthy dogs and no allergy sufferers. Therefore, the already good results of the parameters of faecal quality did not improve. On a high level the protein digestibility rate was improved by about 3%, supporting the hypothesis that protein sources that have been hydrolysed prior to extrusion are in significantly higher rates digested.

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75.

Postprandial amino acid absorption in pigs fed soybean meal or enzyme treated soybean meal

Postprandiale Aminosäurenabsorption bei Schweinen, die mit Sojaschot oder enzymatisch behandeltem Sojaschrot gefüttert wurden

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Greater feed efficiency and balanced diets are strategies for sustainable feeding in pig production and are achievable by improving nutrient digestibility. However, digestibility is a measurement of the degradability of nutrients and only an indirect measurement of nutrient absorption. The aim of the experiment was to show an effect of enzyme treated soybean meal (ESBM) on amino acids absorption in growing pigs compared to conventional soybean meal (SBM).

Method: A total of 7 cross-bred (Danish Landrace, Yorkshire x Duroc) pigs with an average body weight of 18 ± 1.25 kg were fed a commercial diet prior to the study. The pigs were fitted with a jugular vein catheter 3 days before the experimental period. Starting with day one of the trial, the pigs were fed the experimental diet in the morning from day 4 to 5 for 15 min. and received ad libitum of the commercial diet in the afternoon, followed by feed withdrawal after an hour from feeding. Diets were balanced on 20% crude protein. The ESBM (HP 300, HAMLET PROTEIN Denmark) and SBM originated from the same soy batch. Blood samples were collected at time points -30, 30, 60, 90, 120, 180 and 360 minutes after feeding. Plasma was analyzed for individual amino acids. Data was analyzed by the mixed linear model in SAS (version 9.4). The model included time and diet, interaction as fixed effects and pigs included as random effects. Calculation of area under curve (AUC) was according to the trapezoidal method in SAS.

Results: The protein ingredients were fed without addition of free amino acids, therefore the amino acid concentration in plasma represents the amino acid profile, concentration and bioavailability of ESBM and conventional SBM. Until 360 min. after feeding, plasma essential-, non-essential amino acids and lysine in both ESBM and SBM peaked at 60 minutes post-prandial and then slowly declined. The ESBM diet peaked at the concentration of 1.9, 2.1 and 0.23 mmol/L respectively compared to SBM with 1.4, 1.7 and 0.18 mmol/L. The amino acid concentrations were significantly ($P<0.001$) higher for ESBM at 60, 90, and 120 minutes post-prandial compared to the SBM. Since the ESBM and the SBM originated from the same soy batch, the greater bioavailability of ESBM can be ascribed to the processing method as all other variabilities e.g. growing condition of soy, geographic differences and heat treatment can be excluded. From 60 to 360 min. post-prandial, the plasma amino acid concentration in SBM continued to increase slowly and was higher than ESBM at 240 min. post-prandial, which indicates delivery of amino acids to blood plasma after the feeding has finished supposedly due to lower digestibility and absorption rate. The AUC is an indicator of summarized protein digestibility and amino acid absorption over time. The rate of clearance (k_e) indicates how fast amino acids are cleared from plasma. Clearance rate for SBM was negative, possibly meaning that absorption has not ended 6 hours post feeding, or that the increasing amino acid concentration in plasma is a result of muscle protein breakdown. The significantly ($P<0.05$) higher AUC for ESBM indicates higher digestibility and absorption. The contribution to the extracellular pools from the liver could not be determined in the present study.

Conclusion: The postprandial amino acid concentration in plasma of growing pigs was higher in ESBM compared to SBM as measured 360 min. after feeding. The two products originated from the same soy batch, and thus the greater amino acid bioavailability for ESBM can be ascribed to the processing method.

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76.

Effect of increasing condensed tannin intake on fecal and urinary nitrogen excretion in growing finisher pigs

Einfluss ansteigender Aufnahme an kondensierten Tanninen auf den N Umsatz von wachsenden Schweinen

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Condensed tannins (**CT**), a vast class of polyphenolic secondary metabolites, are considered antinutritional factors as they presumably not only reduce feed intake but also impair overall nutrient digestibility and ultimately growth efficiency. It is believed that impaired nutrient digestibility results from the ability of CT to form strong complexes with dietary carbohydrates, protein and minerals. Interestingly, feeding dairy cows CT containing legumes resulted in a significant shift in N-losses from the urinary route to the feces without neither affecting the N-balance nor the milk yield (1). From an environmental point of view, this shift is considered beneficial as ammonia losses from feces occur at a slower rate than from urine. As excessive ammonia losses are an important environmental issue in pig production, the goal of the present study was to monitor to what extent feeding increasing levels of sainfoin to pigs would result in a similar shift in N-excretion as observed in ruminants.

Methods: For the study, a total of 48 Swiss Large White entire males originating from 12 litters and weighing on average 24.8 ± 5.1 kg (mean ± standard deviation) were assigned within litter to 1 of 4 isocaloric (13.5 MJ/kg) and isonitrogenous grower (25-60 kg BW; CP: 16.2%) and finisher (60-105 kg BW; CP: 15.4%) diets. The 4 diets were supplemented with 0 (**T0**), 5 (**T5**), 10 (**T10**) and 15% (**T15**) sainfoin, respectively. For the 2 balance trails carried out during the grower and finisher period, respectively, 6 pigs per treatment were used. Each balance trail consisted of 5 d collection period. Individual feed intake and total amount of urine and feces excreted over the 5 d collection period were recorded. In addition, growth performance and individual feed intake from all pigs were monitored from the start of the trial until slaughter at 109.6 ± 12.6 kg (mean ± standard deviation). Data were analyzed with the MIXED procedure of SAS using litter and experimental groups as fixed effects. Mean differences were tested using the adjusted Tukey test.

Results: Inclusion of sainfoin had no ($P \geq 0.23$) effect on average daily feed intake, growth and consequently feed efficiency. However, despite similar N intake (expressed per kg $BW^{0.75}$), T15 pigs had 32% lower ($P < 0.01$) urinary N excretion compared to T0 and T5 pigs with intermediate values for T10 pigs in the grower period and 39% lower ($P < 0.01$) urinary N excretion compared to T0 and T10 pigs and intermediate values for T5 pigs in the finisher period. In accordance, fecal N excretion was on average 79% greater ($P < 0.01$) in T15 and T10 pigs compared to T0 pigs with intermediate values (+35%) for T5 pigs in the finisher period. Although just a tendency, similar treatment differences were observed also in the grower period. When expressed as percentage of total N intake, urinary N excretions tended ($P = 0.08$) to be 27% lower and were 33% lower ($P < 0.01$) in T15 pigs compared to T0 pigs with intermediate values for T5 and T10 pigs in the grower and finisher period, respectively. Compared to T0 pigs, fecal excretion linearly increased ($P < 0.001$) from +50% to +62% in the grower period and was on average 64% greater ($P < 0.001$) in T15 pigs compared to T0 and T5 pigs in the finisher period. Overall, body N retention was 8% lower ($P < 0.05$) in T5 pigs compared to T0 pigs, with intermediate values for T10 and T15 pigs in the grower period whereas no treatment differences were observed in the finisher period.

Conclusion: The present findings concur with those observed in ruminants as CT from sainfoin cause a distinct shift in N excretion from the urinary route to the feces. However, this shift appears not to have affected overall nutrient availability as growth performance in the grower and finisher period was similar for all dietary treatments. To what extent this urinary to fecal shift in N losses reduces urinary ammonia emission warrants further studies..

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77.

The total tract digestibility of two protein meals (processed poultry meal vs. insect meal from *Hermetia illucens*) in dogs

Verdaulichkeit von zwei Proteinmehlen (processed animal protein poultry vs. Insektenmehl von Hermetia illucens) bei Hunden

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Introduction: The demand for animal protein will increase by 75 % from 2005/2007 to 2050 (1). Therefore alternative protein sources for both, humans and animals, have to be evaluated. The aim of this study was to compare parameters of faecal quality and the total tract digestibility two protein meals in dogs – processed animal poultry (PAPp) on the one hand and insect meal from Hermetia illucens (HI-meal) on the other.
Methods: In a crossover model six adult female beagles (age: 1-3 years) were fed two different diets. The studies were carried out as 'difference trials'. Therefore 30% (of DM) of a purely vegetable basic diet were replaced by both protein sources. The digestibility of the basic diet was already known from other studies (2). By subtraction conclusions about the digestibility of the protein meal could be made. The meals ware characterized as follows:
PAPp per kg DM: OM 853 g, CP 733 g, CF 83.7 g, CA 147 g, Ca 35.2 g, P 23.0 g
HI-meal per kg DM: OM 932 g , CP 570 g, CF 195 g, CA 68.2 g, Ca 11.9 g, P 8.36 g
The insects were reared with feedstuffs for livestock. The amount of feed per day was calculated by the maintenance requirement of 0.4 MJ metabolisable energy/kg bodyweight$^{0.75}$. After an adaption period of 5 days faeces were collected completely for 5 days and the faecal consistency was classified by the following scores: 1 = very hard; 2 = solid, well formed; 3 = soft, still formed; 4 = poor consistence; 5 = watery diarrhea. Furthermore palatability and other faecal parameters like dry matter content and mass were evaluated. Statistical analyzes were done by SAS® (t-test, Wilcoxon-test).
Results and discussion: The palatability of both tested diets was always high even without any adaptation and the intake was never limited or incomplete. There was no difference regarding the faecal mass (g DM/5 days, PAPp 160 ± 14.1, HI-meal 158 ± 9.40). The DM content (%) was significantly higher ($p<0,05$) for PAPp (33.0 ± 2.62 vs. 28.0 ± 2.50), while the consistency of the faeces was significantly more favorable for the HI-meal (2.71 ± 0.577 vs. 2.25 ± 0.375). This might be due to the higher water holding capacity of the HI-meal. The apparent digestibility (%) did not differ significantly regarding the OM (PAPp 86.8, HI-meal 82.5) and the CP (PAPp 83.1, HI-meal 83.4). However the digestibility of CL was significantly higher for the HI-meal (89.1 vs. 95.5), what might be explained by the fatty acid pattern of the insect meals and also by the higher Calcium contents in the PAPp.
Conclusion: The HI-meal showed comparable apparent digestibility rates of OM and CP to PAPp. In case of CF the apparent digestibility was even higher. In addition to high palatability, the consistency of the faeces was more favorable, while the faecal mass did not differ. Taken all together these are important reasons for focusing on insect meal as an alternative protein source in complete feed for dogs. Perspectives of insect meal might be influenced by the price of its production costs, but also by its suitability in dogs reacting on common protein sources with 'food sensitivity'.

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78.

Porcine intestinal cells IPEC-J2 tolerate the absence or elevated levels of L-glutamine and certain environmental stressors

Porzine IPEC-J2 Zellen tolerieren die Abwesenheit oder erhöhte Konzentrationen von L-Glutamin und bestimmten Umweltstressoren

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L-glutamine (GLN) is an important energy source for porcine enterocytes, which show high capacity for its utilisation (1). We hypothesised that different concentrations of L-glutamine in the growth media would divergently influence the performance of the porcine intestinal cell line IPEC-J2 and its response to certain stressors.

Methods: The IPEC-J2 were grown in ThinCertTM cell culture inserts (polyethylene terephthalate capillary pore membranes; 0.4 µm pore size; Greiner BioOne) compatible with 6-well plates (Greiner BioOne). Cells at passage 40 were seeded at a density of 5 x 10^5 cells/well and maintained in the plates during 7 days (37°C; 5% CO_2) changing to fresh medium every second day, until they reached confluency, using the transepithelial electrical resistance (TEER) value as indicator. The growth media consisted of glutamine free Dulbecco's modified Eagle medium (DMEM)/Ham's-F12 supplemented with different concentrations of GLN as follows: none, 2.3 mM (considered a normal concentration), 11.5 mM, 23 mM. In addition, the IPEC-J2 were challenged with environmental stressors e.g. butyrate, H_2S, *Clostridium difficile* toxins A and B. The analyses included TEER, gene expression for certain tight junction proteins, immune markers and antioxidants by rt-qPCR, and cell death using propidium iodide in flow cytometry. The assays were determined in triplicate in three independent experiments. Data were analysed by Friedman test, Kruskal-Wallis test and Mann-Whitney U test with Bonferroni post-hoc corrections, where applicable. Gene expression data were analysed by REST software (Qiagen). The statistical significance was considered at $P \leq 0.05$.

Results: Different concentrations of L-glutamine in the growth media did not affect the TEER measurements ($P > 0.05$). For each single concentration, the TEER was significantly affected by time ($P \leq 0.001$). The viability and gene expression of the cells were not affected by different concentrations of L-glutamine. Neither the addition of butyrate, H_2S, *C. difficile* toxins A and B affected the viability of the IPEC-J2.

Conclusions: We demonstrate that different concentrations of L-glutamine in the growth media do not affect the integrity, viability and gene expression of the IPEC-J2. In addition, epithelial cells may be less vulnerable to certain environmental stress factors.

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79.

Postprandial kinetic of free plasma lysine in dependence of soluble and insoluble protein fractions of feedstuffs for horses

Die postprandiale Kinetik von freiem Plasmalysin in Abhängigkeit von den löslichen und unlöslichen Proteinfraktionen in Futtermitteln für Pferde

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In horses, *in vivo* studies are missing to assess the prececal digestible crude protein (pcdCP) and amino acids (AA) in feedstuffs for horses. Currently, the pcdCP and pcd Lysine (Lys) is estimated in dependence of the neutral detergent insoluble CP (NDICP). *In vivo*, the postprandial changes of free plasma Lys might be a suitable method to evaluate the prececal digestible CP and Lys content. Therefore, the aim of the study was to analyze the postprandial (ppr) Lys responses in plasma after feeding, using diets varying in NDICP values, but being equal in Lys levels. The hypothesis was that the ppr kinetic of free plasma Lys is influenced by the amount of NDICP under the premise of an equal distribution of Lys in the soluble and insoluble protein fractions.

Methods: Three dietary treatments (Lys level of 3.02 g/100 kg body weight (BW)) and one control (1.02 g Lys/100 kg BW) were randomly fed to eight adult Warmblood geldings (617 ± 47 kg BW): a) usual corn-based complementary feed (CF) as control, b) CF plus a synthetic AA containing supplement (SA), c) CF plus soybean meal (SBM) and d) alfalfa pellets (AP). Before feeding and over an 8-hour period postprandial, blood was collected from the *V. jugularis* to analyze the basal level and the ppr kinetic of free plasma Lys. Chemical fractions (CP, NDICP) were analyzed in the feedstuffs (1). The pcdCP was calculated according to GfE (2014) (2). To control whether Lys is equally distributed in the soluble and insoluble protein fractions, Lys in the NDICP residuum was analyzed (1). The pcdLys was calculated according to the estimation equation of the GfE (2014) (2), corresponding to the assumption of equally distribution of Lys in the protein fractions. In comparison to that, a corrected pcdLys content was calculated by using analyzed Lys contents in the soluble and insoluble protein fractions ($pcdLys_{corrected}$). For ppr free plasma Lys, data analysis was performed using analysis of variance factoring for normal distributed data and Wilcoxon signed-rank test for non-parametric data.

Results: The ranking order of the feedstuffs based on pcdCP (in % of dry matter) was: SA 86%, SBM 86% and AP 58%. The calculated pcdLys (in % of Lys content) were: SA 95%, SBM 86%, AP 58%. The numerical increase to maximum ppr free plasma Lys (%, expressed in means ±SD) of SA 100 ±31.4, SBM 100 ±45.3, AP 69.8 ±28.9 developed the same pattern and documented the same ranking order of the feedstuffs *in vivo*. Nevertheless, the analyzed Lys contents in the feedstuffs were not equally distributed over the protein fractions. The $pcdLys_{corrected}$ were: SA 93%, SBM 81%, AP 38%. Assuming that the amount of pcdLys is reflected in the plasma, this estimation of the digestibility of the feedstuffs was confirmed by the numerical increase to maximum ppr free plasma Lys for SA and SBM, but not for AP. Presumably, the chewing process may have an impact on digestibility and in consequence the $pcdLys_{corrected}$ underestimated the Lys digestibility *in vivo*.

Conclusion: Effect of feedstuffs on plasma Lys concentration showed the same ranking as the chemical evaluation, therefore our hypothesis was confirmed. The impact of the chewing process on prececal digestibility of CP and Lys needs further investigations. At present, chemical fractions like NDICP seem to be a suitable method to derive the prececal digestibility of CP and Lys in equine feedstuffs, under the premise of a known Lys distribution over the soluble and insoluble protein fractions.

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80.

Do fruit and vegetable pomaces in varying concentrations affect the *in vitro* ruminal fermentation, methane production and nutrient degradability?

Beeinflussen Frucht- und Gemüsetrester in unterschiedlicher Dosierung Pansenfermentation, Methanproduktion und Nährstoffabbaubarkeit in vitro?

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Food industry by-products such as fruit and vegetable pomaces may serve as animal feed to increase sustainability of meat and milk production. The aim of the present study was to evaluate the suitability of various dried fruit and vegetable pomaces as feed ingredients in ruminant nutrition *in vitro* using the Hohenheim Gas Test method. Furthermore, considering the prevalence of various plant secondary compounds in such residues, their methane mitigation potential, feeding value and effects on rumen fermentation characteristics were evaluated.

Methods: The pomaces (pressing residues) of six fruits (apple (AP), aronia (AR), orange (OR), pomegranate (PG), red (RG) and white grape) and three vegetables (beetroot (BR), carrot (CA) and tomato) were added to a basal diet of hay in concentrations of 15, 30 and 50% of dry matter (DM). Hay without pomace was used as control. The pomaces were tested in duplicates in three runs each, using rumen fluid from a different rumen cannulated cow (n = 3) in each run. Incubation procedure was according to Menke and Steingass (1) and included the calculation of the *in vitro* organic matter digestibility (IVOMD). Data were analysed using the Mixed model of SAS (version 9.3) considering the treatment as fixed effect, and animal and run as random effect.

Results: The crude protein content of pomaces ranged from 2.19 (BR) to 12.31% (PG), that of neutral detergent fibre from 21.02 (OR) to 87.16% (CA). The content of total extractable polyphenols ranged from 0.21 (CA) to 10.91% (PG). The pH of the rumen fluid ranged between 6.81 and 6.99. Methane formation was only affected by CA and BR. Incubation with 50% of CA increased the absolute methane volume by 4 mL/24h ($p = 0.002$), the methane per g DM by 20 mL ($p = 0.002$) and by 22 mL ($p = 0.006$) per g digestible organic matter (dOM) when compared to the control. Likewise, 50% of BR increased methane per g dOM compared to the control by 18 mL ($p = 0.048$). Ammonia concentration was lower after incubation with 50% of AP compared to the control (4.8 vs. 7.2 mmol/L; $p = 0.038$). The other pomaces had no effect on ammonia concentration. Despite this small number of significant differences when treatments were compared to the control, many significant differences were observed when pomaces were compared among each other. The total fermentation gas volume was higher with 50% of AP compared to incubation with PG and RG in all three concentrations tested as well as when compared to 15 and 50% of AR (up to 19.6 mL/24h; $p < 0.05$). Absolute methane production, methane per g DM and per g dOM were reduced ($p < 0.05$) by incubation with 50% of both AR and PG when compared to 50% of CA (reduction of up to 5.7 mL/24h, 28.7 mL/g and 27.3 mL/g). These results are most likely due to the reduced IVOMD with 50% AR and PG compared to CA (IVOMD in mg/g: 55.7 and 58.9 vs. 74.2; $p < 0.05$) and the likewise reduced dOM (in mg/day: 113 and 117 vs. 144; $p < 0.05$). The decrease in digestibility with AR and PG compared to CA may have been caused by high amounts of polyphenols in both AR and PG on the one hand and high amounts of sugars in CA on the other hand. The observed decreases in IVOMD and dOM when comparing 50% to 30% AR incubation (55.7 vs. 69.8 mg/g; $p = 0.015$ and 113 vs. 141 mg/day; $p = 0.028$) supports the assumption of decreased digestibility with increasing concentration of polyphenols. Bacteria and protozoa counts did not significantly differ between treatments.

Conclusions: The preliminary results of the present study suggest that most of the tested pomaces could potentially be fed to ruminants in concentrations of up to 50% of DM of forage only diets without negatively affecting rumen fermentation. Despite an apparent lack of methane mitigation potential, the high polyphenol contents in pomaces from AR, PG and RG make them interesting feed ingredients to be investigated with regard to their potential benefits for animal health and the quality of meat and milk *in vivo*.

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81.

Effect of finely-graded differences in dietary trypsin inhibitor activity on zootechnical performance and pancreas development of broilers

Zum Einfluß feinabgestufter Unterschiede in der Trypsininhibitoraktivität der Gesamtration auf die zootechnische Leistung und die Entwicklung des Pankreas im Mastgeflügel

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Introduction. The efficiency by which the feed matrix is degraded within the gastrointestinal tract significantly determines the efficiency and rentability of animal production systems. Residual activities of trypsin inhibitors in feedstuffs may impair the feed conversion ratio in a significant manner. Different authors suggest varying thresholds of trypsin inhibitor activity (TIA) in soybean products ranging from 0.5 to 4.7 mg/g below which no adverse effects on animal productivity should occur (1-3). This study aimed at challenging broilers with finely-graded differences in TIA to test these thresholds.

Methods: This study comprised 1680 male ROSS 308 broilers (Aviagen Group), which were used during two consecutive runs (840 birds/trial) due to limited total stable capacity (each run included 105 cages of 8 birds/cage). After a starter phase of 10 d during which all birds received a commercial starter diet animals were assigned to 35 different treatment groups (6 cages/group; 48 birds/group). These groups received diets during grower and finisher period containing 35% and 25%, respectively, of one of 34 differentially treated (thermal, hydrothermal, pressure, kilning) soybean cake variants (TIA ranges grower: TIA of 0.5-8.7 mg/g; TIA ranges finisher: 0.3-7.2 mg/g). Additionally, one group received commercial soybean meal as a control (dietary TIA: 1.2 mg/g). Apart from the soybean products, diets consisted of corn, wheat, soybean oil and a premix (minerals, vitamins) to meet or exceed current feeding recommendations for ROSS 308 broilers. All birds had access to feed and water *ad libitum* at all stages of the trial. Data collection consisted of life weight development of individual birds at d 1, 10, 24 and 35 as well as total per cage feed intake during grower and finisher phase, respectively. Furthermore, birds were slaughtered at d 35 and the pancreas weight of each individual bird was determined. Statistical analysis comprised linear multifactorial (TIA, reactive lysine, KOH-soluble crude protein) regression models with SAS 9.4 (SAS Institute Inc.).

Results: Total weight gain (TWG), total feed intake (TFI) and live weight significantly declined during grower and finisher phase, respectively, inversely to the dietary TIA level ($p < 0.01$). In contrast, feed:gain ratio at the end of the grower phase correlated directly and significantly to the dietary TIA level ($p < 0.0001$) but was not different between groups at the end of the finisher phase ($p = 0.44$). The pancreas weight was directly associated to dietary TIA in a significant manner ($p = 0.03$). The dietary amounts of reactive lysine and KOH-soluble crude protein did not express any significant effects on the zootechnical performance or pancreas development whatsoever.

Conclusion: Soybean processing jointly modified dietary TIA, reactive lysine and KOH-soluble crude protein. However, only TIA negatively affected animal performance and pancreas development in a significant manner. The effectiveness of TIA was given over the whole range of dietary activities including very minor activities far below published thresholds. It can be concluded that a significant elimination of dietary TIA is highly recommended under practical feeding conditions when generous safety margins are applied in terms of the supply with essential amino acids especially lysine. A specific TIA threshold for final feed mixtures cannot be recommended due to a strictly linear response of the assessed zootechnical and pancreatic measures.

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The project was supported by funds of the German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the German Federal Office for Agriculture and Food (BLE) under the protein strategy program (FKZ: 2814EPS022).

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82.

Feeding two separate meals of crushed or pelleted oat grains from different varieties and the effect on postprandial blood concentrations of amylase, glucose, GLP-1 and insulin in adult healthy horses

Fütterung von zwei Mahlzeiten von gequetschtem oder pelletiertem Hafer verschiedener Sorten und dessen Effekt auf die postprandiale Blutkonzentration an Amylase, Glukose, GLP-1 und Insulin im Blut von gesunden Pferden

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Genetic diversity of oats seems to modify the postprandial (ppr.) glycaemic and insulinaemic response to oat grains (OG) in horses particularly strong (1). However, data is missing comparing the first and second meal effect in this concern. With other starchy feeds, the impact of the second meal seems less pronounced (2, 3). Although intense processing of OG is not necessary to achieve a high precaecal starch digestibility, gentler procedures like crushing (CRU) or pelleting (PEL) were less noticed. Aim was to compare effects of 2 meals/d of 1 g starch/kg body weight (bwt) each from OG of 3 genotypes, fed native (NAT), CRU or PEL, on ppr. plasma responses of amylase, glucose, glucagon-like peptide 1 (GLP-1) and insulin.

Methods: 6 mares (age 11.3 ± 2.65 years; bwt 532 ± 35.6 kg; body condition score 5.2 ± 1.3/9) got in 2 equal meals/d (7 am/2 pm) 1.5 kg hay/100 kg bwt d^{-1} and 1 g starch/kg bwt $meal^{-1}$ either from NAT, CRU or PEL (diameter 3 mm) OG of 3 genotypes ('Bison', 'Scorpion' and 'Yukon': 506, 474 and 479 g starch/kg dry matter). The adaptation period lasted for 3 weeks and for each case (3 genotypes x 3 treatments; cross over) blood was sampled at the end of the 7 d main period. Blood sampling occurred 1 h following feeding of 1.5 kg of hay (t_0), then OG were given and blood sampled 15, 30, 60, 90, 120, 180, 240, 300 min afterwards. Plasma amylase (COBAS 311), GLP-1 (active ELISA, IBL, Hamburg), glucose (Hitachi 912) and insulin (Insulin-ACount-RIA-Kit) were determined and the area under the curve (AUC) calculated. For this, the area above basal ($t_{0;}$ meal 1 *vs*. meal 2) was calculated as positive ($AUC_{(gluc,\ ins,\ GLP\text{-}1)+}$) and the area below t_0 was calculated as negative $AUC_{(gluc,\ ins,\ GLP\text{-}1)}^{-}$. Finally both were summed up. For statistical analysis, MIXED model was used (SAS 9.4).

Results: Meal 1 *vs* 2 induced higher ($P < 0.05$) AUC_{gluc} (295 *vs* 56 mmol/L min^{-1}), $AUC_{GLP\text{-}1}$ (1,907 *vs* 403 nmol/L min^{-1}) and AUC_{ins} (155 *vs* 13 nmol/L min^{-1}), which coincides with a higher overall mean of amylase ($P < 0.05$). With native OG, the highest AUC_{gluc} (mmol/L min^{-1}) and AUC_{ins} (nmol/L min^{-1}) were observed for `Bison` followed by `Yukon` and `Scorpion` (meal 1: 381 and 189; 280 and 119; 203 and 119; meal 2: 74.8 and 7.7; -17.1 and 1.8; -27.9 and 6.3). Crushing had no impact, but pelleting did (meal 1: 353 and 141; 219 and 141; 384 and 203; meal 2: 130 and 31.6; 78.7 and 22.6; 65.0 and 8.0). The highest $AUC_{GLP\text{-}1}$ (nmol/L min^{-1}) was calculated for crushed `Bison`, followed by `Yukon` and `Scorpion` (meal 1: 2,460; 2,338 and 2,276; meal 2: 1,064; 1,374 and -151) compared to native OG but most notably relative to pelleted OG (meal 1: 1,107; 1,511; 1,843; meal 2: -449; 518; -287).

Conclusion: The first oats meal per day induces particular high plasma responses of amylase, glucose, GLP-1 and insulin. Crushing and pelleting seem not to elevate glucose availability from starch in general, but there may be an interaction between processing and genotype. Pelleting increased amylase combined with particular low GLP-1 concentrations. Maybe only small amounts of glucose passed the terminal small intestine and the large bowl were GLP-1 releasing L-cells are located. This would be the case if the starch from pelleted OG was particularly high available in the foregut. However, the glycaemic response does not support this assumption. It should thus be consider that pelleting might facilitate the microbial fermentation of starch in the stomach.

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The study is part of the project 'GrainUp' and was supported by the German Federal Office for Agriculture and Food (BLE).

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83.

Using soluble and insoluble dietary fibre in piglet nutrition – Effect of soybean hulls and lignocellulose on performance and physiology

Einsatz von löslicher und unlöslicher Faser beim Absetzferkel- Einfluss von Sojaschalen und Lignocellulose auf die Leistung und Physiologie

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Dietary fibre is a very heterogeneous and not well understood nutrient in monogastric animal nutrition but is getting more attention due to its fundamental properties throughout the whole gastro intestinal tract. Physiologically it is the part of carbohydrates which is not digested by enzymes of the intestine but partly fermented by the intestinal microbiota, especially in the hindgut (1). Thereby the differentiation between soluble and insoluble part of fibre may influence the prediction of nutrient digestibility (viscosity), concentrations of biogenic amines or SCFA in the hindgut (fermentability) and further the immunological cascades in specific tissues. Therefore the objective of the present study was to investigate the effect of soluble and insoluble dietary fibre on animal performance, apparent total tract digestibility (ATTD), concentration of microbial metabolites in colon and ileum chyme as well as proinflammatory marker genes in piglets. It was hypothesized that the variation of insoluble fibre may promote beneficial effects regarding the development stage of piglets' gut compared to soluble fibre proportion.

Methods: Two consecutive feeding trails with 48 weaning piglets (28 days old) each were conducted. The animals were randomly allotted to one of four treatments: T1 with no specific fibre source (barley), T2 included soybean hulls (2.5%), T3 and T4 lignocellulose I (high fermentable, 1.5%) and II (low fermentable, 1.5%). The animals were housed in eight conventional pens, with two pens per treatment, each six piglets. To ensure that selected fibre sources were composed of different fractions of soluble and insoluble dietary fibre, the amount of fibre was equalized on the basis of total dietary fibre (150 g/kg TDF) for each treatment. A starter diet was fed the first 14 days followed by grower diet until experimental day 54. Metabolisable energy and protein content were balanced calculated for the different treatments within starter (13.6 ME, 20% CP, 15% TDF) and grower period (13.5 ME, 22% CP, 14% TDF). Bodyweight of piglets was determined at the beginning of the trial and then continuously every week until the end of experiment in week eight for average daily gain (ADG) calculation. Feed consumption per pen was recorded weekly to calculate average daily feed intake (ADFI) and FCR. Feaces were collected in the 7th week individually for calculation of ATTD of DM, CP, aNDFom, CA and EEh. Piglets of the first trial were slaughtered followed by sampling of tissue (lymph nodes, liver, spleen, ileum) and digesta (colon and ileum) were prepared for molecular as well as for analysis of microbial metabolites. Samples of feed and fibre sources were taken for proximal analyses (DM, CP, CA, EEh, CF, starch) and fibre content (aNDFom, ADFom, ADLom, SDF, IDF, TDF). Statistical analyses were performed by ANOVA (GLM procedure) of SAS (SAS Inst., Inc., Cary, NC, USA). Means were compared using the least-squares means statement and differences were determined using the Tukey-Kramer test with values of $p<0.05$ stated as significant.

Results: The moderate/minor variation of soluble or insoluble fibre sources between treatments had no effect on animal performance over the whole trial period ($p>0.10$). Feeding soybean hulls increased ($p<0.05$) the ATTD of aNDFom, OM, DM, and EEh which might be explained by the higher amount of fermentable compounds within this fibre source. Analyses of biogenic amines revealed significant differences for the concentration of cadaverin (ileum, lowest in T4 and highest in T2) while no changes in content of SCFA occurred. Even the proinflammatory marker gens (TNFa, IL8, NfkB) did not differ between the treatments in analysed tissues.

Conclusion: This study revealed minor effects by moderate/minor variation of soluble and insoluble dietary fibre in diets of weaning piglets. The main reason for these observations might have been based on the low inclusion level of fibre source to clarify the mode of action.

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84.

Feed processing effects on energy digestibility and performance of broilers

Effekt der Futterverarbeitung auf die Energieverdaulichkeit und zootechnische Leistung von Masthühnern

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In a diet based on corn and soybean meal for broilers, corn has quantitatively the biggest effect on the particle size regulation of the compound diet. In broilers, coarse particle size is associated to higher digestibility of the organic matter by promoting a higher development and activities of the gizzard (Naderinejad et al., 2016). This results in longer retention time, lower pH, more refluxes cycles, and consequently improved digestive enzymatic hydrolyzes reactions, resulting in higher absorption rates of nutrients. Typically, two mills are used for grain grinding, i.e. hammer mill (HM) and roller mill (RM). In addition, adjustments in the feed processing line such as the hydrothermic treatment with expander prior to pelleting may improve feed hygienization, nutrient digestibility and reduce thermo-labile anti-nutritional factors in compound feeds (Puntigam et al., 2017). However, studies establishing relationships between mill type, particle size and conditioning prior to pelleting are scarce. Therefore, the objective of the current study was to evaluate the effect of using three adjustments in the feed processing line on energy digestibility. We hypothesized that the combination of coarse grinding with the expander processing prior to pelleting improve the energy digestibility.

Material and Methods: a total of 864 one-day-old unsexed broiler chicks Ross 308 were randomly allocated to 36 pens with 12 birds each in a two periods trial, at the poultry research station 'Wimitz' in Carinthia, Austria. Water and feed were available *ad libitum*. Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were measured at 38 days of age. Corn-soybean meal diets were formulated based on Austrian commercial practices. Until day 8, birds received a common diet (AMEn: 12.35 MJ/kg and dig. Lys: 1.16%). Two feeding phases were used, the grower was from 8 to 22 days (AMEn: 12.80 MJ/kg and dig. Lys: 1.05%) and the finisher from 23 to 38 days (AMEn: 12.70 MJ/kg and dig. Lys: 0.83%). Experimental diets were processed with different adjustments to obtain 12 treatments. Either a HM or a RM was used. In between these two mills, three different particle sizes were produced, using corn, soybean meal and grass meal mixed following the formulation. Half of each batch was afterwards conditioned and pelleted, while the other half was conditioned and expanded prior to pelleting. This resulted in a 2x3x2 factorial arrangement. Statistical analysis was performed, and differences accepted when $P<0.05$.

Results: In the overall period, FI was affected just by expander, with lower intake (2%) by the birds fed with diets with expander. This effect was extended to FCR as lower intake resulted in better feed efficiency (1.94%), at a similar BWG (2.45kg). The energy digestibility shows no differences for mills (HM=0.76, RM=0.74), but was affected by particle size, i.e. it was higher for 1.2 mm (0.77) in comparison with 1.6 mm (0.72). In the same way, the digestibility was better with expander (0.77) compared to not expander (0.73). This resulted in different AMEn for expander and particle size factors, with 15.30 MJ with expander and 14.90 MJ without and 15.40 MJ with 1.2 mm and 14.80 MJ for 1.6 mm, respectively. At slaughter, abdominal fat was higher after expander processing. With expander, the abdominal fat proportion was 1.60% with and 1.50% without Expander.

Conclusion: Using the expander promotes higher AMEn, confirmed by the higher proportion of abdominal fat content. Therefore, it is important to consider processing of diet formulation for AMEn balancing. Results suggest a reduction of 3% in AMEn from ingredients, this energy is provided by the expander.

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85.

Are there differences in starch gelatinisation between differently processed laboratory rodent maintenance feedstuffs marketed as identical?

Bestehen Unterschiede zwischen dem Stärkeaufschlussgrad als gleichartig vermarkteter Erhaltungsfuttermittel für Labornager in unterschiedlicher Konfektionierung?

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Processing alters the structural properties of starch as well as its praecaecal digestibility. Yet maintenance diets for laboratory rodents are often marketed both as pellets and extrudate labelled as the same diet with identical analytical constituents. This might prove to be problematic in terms of animal experiment standardisation and the 3Rs (1). Factors beyond the labelled nutrients, such as starch gelatinisation, should be taken into account when designing an experiment and interpreting the Results: Up to now, this is not commonly acknowledged by manufacturers and researchers who are not primarily nutritionists. The aim of the present exploratory study was therefore to compare the degree of starch gelatinisation of different laboratory rodent feedstuffs for maintenance. The hypothesis was that there would be differences in starch gelatinisation between pelleted and extruded feedstuffs labelled as the same diet.

Methods: Laboratory rodent maintenance diets (n = 11) of three different brands were acquired. Of each brand, differently processed products (pellets n = 6, "hybrid pellets" n = 1, extrudate n = 4; some also sterilised) were used that were labelled as the same diet in different confection. Starch gelatinisation of each feedstuff was measured according to the VDLUFA method.

Results: In general, the percentage of gelatinised starch was significantly higher in the extruded diets than in pellets (64.8 ± 4.2% vs. 18.6 ± 5.2%, mean ± SD, $p<0.001$). The starch gelatinisation was 13.0% in the pellets and 58.6% in the extrudate of brand I. Samples of the same products that had been autoclaved showed starch gelatinisation grades of 18.4% and 65.7%, respectively. In brand II, so-called hybrid pellets had a percentage of starch gelatinisation in-between pellet and extrudate of the same brand (43.1% vs. 20.4% and 68.0%). In brand III, pellets ranged between 12.0 (not sterilised) and 24.1% (sterilised) while the extruded maintenance diet had a starch gelatinisation of 66.9%.

Conclusion: The results confirm the hypothesis that there are vast differences in the degree of starch gelatinisation in laboratory rodent feedstuffs labelled as equal but differing in type of processing. The extruded diets had a significantly higher degree of starch gelatinisation than the pellets, as could be expected. Additional treatment also influenced starch gelatinisation. Praecaecal starch digestibility is altered by processing. The amount of praecaecally undigested starch that passes into the large intestine influences the intestinal microbiome in rodents as well as other species, including humans (2). It is therefore important to standardize or take into account the starch gelatinisation grade when interpreting or comparing studies in which differently processed diets have been used, even when the diet itself was not the main focus of the experiment.

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86.

Effect of lactic acid treatment of cereals and dietary phytase on fecal microbiome and shedding of virulence factors in growing pigs

Einfluss einer Behandlung von Getreide mit Milchsäure und Phytasezusatz auf das fäkale Mikrobiom und die Ausscheidung von Virulenzfaktoren beim wachsenden Schwein

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Uncovering effects of dietary changes on the gut microbiota is of great interest in order to maintain high gut health and well-being in pigs as well as to reduce fecal shedding of opportunistic pathogens associated with zoonotic diseases (1). Besides the major nutrients, phosphorus is an essential for both the host animal and the porcine gut microbiota. Different strategies are used to enhance the phosphorus availability from cereals in pig diets, such as supplementation of phytase or soaking of cereals (2). However, their impact on the gut microbiota has been rarely related to virulence factor shedding in feces. Therefore, this study examined the effect of lactic acid treatment of dietary cereals and phytase supplementation on fecal microbiome of metabolic active bacteria and fecal shedding of virulence factor genes.

Methods: Thirty-two castrated male pigs (Large White, 13.2 kg, 6-8 weeks of age) were fed one of four wheat (36.2%)-corn (36.0%)-soybean meal based diets according to a completely randomized design with 4 replicate batches: control diet (Con), diet with phytase (500 FTU/kg; Phy), diet containing lactic acid-treated cereals (LA), and diet with phytase and lactic acid-treated cereals (LA-Phy). For LA and LA-Phy diets, cereals were soaked in 2.5% LA for 48 hours at 21°C, dried at 70°C for 1 hour and afterwards at 60 °C for 23 hours. Pigs were individually housed in metabolism pens and fed 3-times daily. Feed allowances corresponded to 3-times of pig's maintenance requirement. Each experimental period lasted 19 days. On day 19 feces were collected for total RNA isolation. After cDNA synthesis, the V3-V4 region of the 16S rRNA gene was sequenced on an Illumina MiSeq platform. Abundances of virulence genes from *Clostridium perfringens* and enterotoxigenic *Escherichia coli* were determined using quantitative PCR. Bioinformatics was performed in QIIME. Bacterial data were analyzed by ANOVA using the PROC MIXED procedure in SAS with the model of the fixed effects of replicate and treatment effect, considering significant difference at $p < 0.05$. and trends at $0.05\ p < 0.10$.

Results: Total bacterial gene copies in feces were similar among dietary treatments. Phytase decreased the relative abundance of the most dominant family *Lactobacillaceae* ($p<0.05$) and, as trend ($p<0.10$), less abundant *Enterobacteriaceae,* whereas the second most abundant family *Clostridiaceae* was increased by both phytase supplementation and LA-treatment of cereals ($p<0.05$). The LA-treatment of cereals further depressed amylolytic and hemicellulolytic *Lachnospiraceae* and *Ruminococcaceae* ($p<0.05$). Although phytase affected the genera *Clostridium* and *Escherichia*, fecal excretion of heat-stable toxin A (Sta) and Shiga toxin Stx2e from enterotoxigenic and Shiga-toxin producing *Escherichia coli*, respectively, was not different among treatment groups. Moreover, α- and β-toxin genes from *Clostridium perfringens* were barely detectable in feces.

Conclusions: The present findings demonstrate that, without modifying the absolute abundance of metabolically active bacteria, both lactic acid treatment of cereals and phytase supplementation drastically altered the fecal bacterial composition. By contrast, shedding of virulence factor genes from pathogenic *E. coli* and *C. perfringens* in feces were unaffected by diets. Although altered bacterial abundances can be related to the enhanced intestinal P availability with phytase supplementation, changes in the dietary starch and hemicellulose fractions in the cereals due to the LA treatment may have contributed to the observed effects as well.

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87.

Crushing or pelleting of oat grains from different varieties and the effect on the morphology of starch granules and chewing parameters in horses fed twice per day

Quetschen oder Pelletieren von Hafer verschiedener Hafersorten und dessen Effekt auf die Stärkemorphologie sowie Kauparameter des Pferdes bei zweimaliger Fütterung pro Tag

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Several processing methods modify not only the visual appearance of cereal grains but also morphological properties of their starch granules (1). Chewing patterns during ingestion and the enzymatic degradation can thereby be influenced (2). The first, however, mainly if physical characteristics of the feed *per se* are altered (e.g. by pelleting). Beside the impact of feed processing, until now there was no investigation if mealtime feeding has an impact on feed intake characteristics. By the example of oat grains (OG) as a particular typical cereal source for horse feeding, the impact of oat variety and feed treatment (crushing, pelleting) on the morphology of starch granules as well as on feed intake patterns should be investigated when the OG are given in two equal meals per day.

Methods: Six warmblood mares (age 11.3 ± 2.65 years; body weight [bwt] 532 ± 35.6 kg; body condition score 5.2 ± 1.3/9) got in 2 equal meals/d (1 g starch/kg bwt meal^{-1}, 7am/2pm) either native (NAT), crushed (CRU) or pelleted (PEL, diameter 3 mm) OG. Three different OG varieties ('Bison', per kg dry matter [dm]: 509 g starch, 126 g crude protein [CP], 36 g crude lipids [CL], 155 g acid detergent fiber [ADF], 12.9 MJ ME; 'Scorpion': 474 g starch, 127 g CP, 44 g CL, 167 g ADF, 13.3 MJ ME; 'Yukon': 479 g starch, 131 g CP, 41 g CL, 153 g ADF, 13.5 MJ ME) were allocated according to a cross over design with period length of 7 d. Additionally meadow hay was fed at 1.5 kg/100 kg bwt d^{-1} (per kg dm: 54 g CP, 7 g CL, 346 g ADF, 8.3 MJ ME). On d7, similar for both mealtimes 1.5 kg of hay was fed 1 h prior to OG. Feed intake time (FIT, in min/kg dm) and count of chews (CC) were measured by modified halters (2) and analyzed for chewing frequency (CF, in CC/s) and chewing intensity (CI, in CC/kg dm). Electron microscopy was used to characterize morphologic properties of starch granules and their matrix structures (x 1500). Mixed model (fixed factors: mealtime, treatment) was used for statistical analysis (SAS 9.4).

Results: Starch granules and matrix substances lost their original structure and merged after crushing and even more pronounced after pelleting. 'Yukon' *vs* 'Scorpion' and 'Bison' (CI: 909 *vs* 1029 and 1004; $P < 0.05$), and PEL *vs* CRU and NAT ($P < 0.05$) reduced CI (898 *vs* 1034 and 1010) and FIT (10.0 *vs* 11.3 and 11.5). Meal 1 *vs* 2 took longer to be ingested (FIT: 11.6 *vs* 10.4; $P < 0.05$) with reduced CF (1.44 *vs* 1.47; $P < 0.05$) but higher CI (1021 *vs* 940; $P < 0.05$).

Conclusion: Irrespective of the OG treatment, the first meal per day was eaten slower and chewed more intensely than meal 2, whereas both OG meals had the same forewent hay meal. Pelleting caused a rapid feed intake and reduced chewing intensity as an overall effect what confirms previous results (2). Whether the intensive merging of starch granules after pelleting indicates rather starch gelatinization or retrogradation, with accordingly opposing effects on the postprandial glycaemic and insulinaemic response needs to be investigated.

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The study is part of the project 'GrainUp' and was supported by the German Federal Office for Agriculture and Food (BLE).

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88.

Variation of the hygienic status of hay due to soaking and steaming as well as subsequent storage at different temperatures

Veränderung des Hygienestatus von Heu durch Wässern und Bedampfen sowie nachfolgender Lagerung bei unterschiedlichen Temperaturen

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Hay plays an important role in horse feeding but due to the weather-dependent nature of hay production, the feed is prone to an insufficient hygienic quality caused by microorganisms, which in turn might lead to health problems in horses (1). Soaking and steaming are known to improve the hygienic quality (2), but little is known on the impact of subsequent storage. Particularly soaking is also used to reduce water-soluble carbohydrates (WSC) in the hay. Aim was to study the impact of soaking and steaming on WSC in the hay and also the treatment and storage effect on its hygienic quality.

Methods: Native meadow hay (NATIV; 1st cut, end of blossom) from one batch was either soaked (SOAK) on model scale (for 15 min, then drained for 10 min) or steamed (STEAM; 60 min at a temperature of greater than 80 °C; haysteamer HG one+, Haygain, Farm & Stable, West Sussex, UK) and subsequently stored for different times (6 h, 12 h and 24 h), in each case at two different temperatures (10 °C, 25 °C). The hay was analyzed for water soluble carbohydrates (WSC: glucose, fructose, sucrose, fructans; HPLC) and microbial counts (in colony forming units [CFU], [3]). ANOVA (SAS 9.4) was used for statistics.

Results: WSC in the hay decreased following soaking or steaming (0 h: NATIV 14.1 % dry matter [DM] *vs* SOAK: 6.7 % DM/STEAM: 8.1 % DM; $P < 0.05$; SOAK vs. STEAM; $P < 0.05$) and furthermore during storage. NATIV contained 29 x 10^6 CFU/g typical bacteria, 64 x 10^3 CFU/g typical fungi and 102 x 10^3 CFU/g yeasts, but no spoilage indicating microorganisms, meaning that the hygienic quality was within recommended benchmarks (3). SOAK *vs* NATIV reduced typical fungi (50 x 10^3 CFU/g *vs* 64 x 10^3 CFU/g; $P < 0.05$), but they increased again with storage time ($P < 0.05$). In STEAM, no typical fungal species were detected. Spoilage-indicating fungal species were only determined in SOAK. No yeasts were detected in STEAM but SOAK, and they were determined in increasing numbers with storage time and temperature (0 h: 90 x 10^3 CFU/g to 24 h: 545 x 10^3 CFU/g) which exceeded the recommended benchmark (150 x 10^3 CFU/g, [3]).

Conclusion: The native hay investigated here was of good hygienic quality (3). Both treatments decreased the content of WSC, soaking to a higher extend than steaming. Furthermore, the soaking of hay forced the formation of the spoilage fungi *Mucorales* already after the treatment (0 h) which in turn might provoke the development of zygomycosis. Long storage time (≥ 12h) of soaked hay in association with warm temperatures amplifies the proliferation of spoilage-indicating molds/yeasts which might forces the development of digestive problems (1). In opposite, steaming was very effective concerning the reduction of yeasts (2) and the steamed hay thus less susceptible to spoilage induced alterations. Generally, both treatments are very effective concerning the reduction of respiratory diseases caused by alveolar particles.

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89.

Effect of different pre-ensiling treatments on *in vitro* ruminal fibre degradation of lucerne silages

Einfluss verschiedener Behandlungen vor der Silierung auf den ruminalen Faserabbau von Luzernesilagen in vitro

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Preserving lucerne (*Medicago sativa* L.) as silages has the aim to minimize nutrient losses during ensiling and to produce a high-protein forage for dairy and beef cattle. At ensiling, pre-ensiling treatments like different wilting intensities (high or low), dry matter (DM) levels (25% or 35%) and sucrose addition (with or without) modulated the *in vitro* ruminal fermentation pattern of lucerne silages (LS) with increased isovalerate concentrations during incubation of sucrose-added LS (2). As isovalerate is a branched-chain short-chain fatty acid (SCFA), which stimulates predominant rumen cellulolytics and is crucial for their growth (1), we hypothesized increased fibre degradation in this LS type when compared to other LS that received different pre-ensiling treatments. Thus, degradation of fibre fractions was determined three and eight days after first-time incubating LS into the *in vitro* Rusitec system. Here, we also hypothesized higher fibre degradability at the second determination due to a better adaption of the microorganisms to the LS.

Methods: The LS were tested in a Rusitec system consisting of six vessels, where the silages were randomly incubated in quadruplicate during six consecutive runs. Three and eight days after first-time LS incubation, daily gas production was measured and samples for SCFA analysis were taken 2, 4, 12 and 23 hours (h) after feed bag exchange. In addition, 48-h feed bag residues were collected for subsequent determination of neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom) and acid detergent fibre expressed exclusive of residual ash (ADFom). The four SCFA samples were pooled for each vessel and run, respectively, to obtain robust mean values. Analysis of SCFA was performed by gas chromatography (GC Auto System, Perkin Elmer Inc., Waltham, MA, USA), gas production by water displacement technique and fibre fractions were determined according to standard procedures of VDLUFA. Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with sucrose addition, wilting intensity, DM level and sampling day as fixed effects and vessel and run as random effects.

Results: For the first sampling day, sucrose-added LS with 25% DM had highest aNDFom degradability, which supports our hypothesis of greater fibre degradability with increased isovalerate concentration. However, this correlation was not true for ADFom and, although isovalerate concentration increased during incubation of sucrose-added LS, ADFom degradability was higher for LS without sucrose addition for both sampling days.

Regarding the two sampling days, isovalerate concentration increased from an average of 7.1 mmol/L to 9.2 mmol/L, whereas degradability of aNDFom and ADFom decreased between the sampling days from an average of 38.1% to 32.5% and 37.7% to 28.5%, respectively, which falsifies our hypotheses of a fibrolytic stimulation by isovalerate and also of a better microbial adaption to LS. Interestingly, reduction of fibre degradability was accompanied by a 2.8 mmol/L decrease in n-butyrate concentration, but the acetate concentration increased by 2.9 mmol/L from day three to eight. Thus, the SCFA profile does not explain the fibre degradation values. However, acetate can also originate from ruminal deamination, which should be considered as possible reason.

Conclusions: Low DM may have a decreasing effect on silage quality; however, ruminal degradabilities of fibre fractions, i.e. aNDFom and ADFom, may be positively affected. The role of isovalerate for fibre degradation seems less clear and partly contradictory. Analyses of microbial abundances, their composition as well as activated metabolic pathways may help to elucidate the underlying mechanisms and the over time decreasing fibre degradability.

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90.

Effects of toasting temperature and time on ruminal gas production kinetics and post-ruminal crude protein from field pea (*Pisum sativum*) grain silages measured *in vitro*

Effekte von Temperatur und Dauer des Toastens silierter Erbsenkörner auf die Gasbildungskinetik im Pansen und das nutzbare Rohprotein am Duodenum in vitro

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Ensiling and subsequent toasting of field pea (FP) grains might partly protect starch and protein against ruminal degradation. The aim was to investigate this *in vitro* after applying variable toasting temperatures and times during model scale treatments.

Methods: Model silages (1) were prepared from re-moistened FP grains (~ 71 % DM) using a *L. plantarum* LSI NCIMB 30083 inoculant (6.8×10^6 CFU/g fresh matter). Aerobic stability was tested after 60 d. Toasting of ensiled FP was simulated in a drying oven. First, temperature was varied from 120, 140, 160, 180 up to 200 °C, time was kept constant at 30 min (experiment A). Secondly, time of toasting was varied with 10, 20 and 30 min at constant 160 °C (experiment B). For *in vitro* incubation, the ANKOM^{RF} Gas Production System was used. In 6 trials per experiment rumen fluid was obtained from 2 rumen-cannulated adult Suffolk wethers. The inoculum was prepared according to VDLUFA (2), but we added NH_4HCO_3 by 2 g/L and reduced $NaHCO_3$ by 2 g/L to avoid limiting N availability. In each vessel, 0.2 g of pulverized substrate and 30 mL of inoculum were incubated at 39 °C and 80 rpm. Cumulative gas pressures were automatically recorded over 24 h, blank corrected, and converted to mL of gas produced (7–12 replicates per treatment). After 8 and 24 h, samples were taken for NH_3-N analysis (8–12 replicates per treatment each), and post-ruminal CP (PRCP) was calculated. Rumen-undegraded protein (RUP) was estimated using the *Streptomyces griseus* protease test (4 replicates per treatment at 8 or 24 h) (3). Effective PRCP and effective RUP were calculated for assumed rumen passage rates (*K*p) 0.02, 0.04, 0.06, 0.08 and 0.12/h. Scanning electron micrographs (SEM) of starch granules were recorded to visualize morphological changes in response to the treatments. Starch was determined using the amyloglucosidase method (2). True protein (TP) and protein solubility (PS) were calculated based on analyses of protein fractions. Pepsin-insoluble protein (PIP) was analysed by Kjeldahl after 48 h of incubation in a pepsin-hydrochloric acid solution. Organic acids and ethanol were determined in silages by HPLC and refractive index detection. Statistical analysis was performed using SAS 9.4 MIXED. Non-linear regression analysis was performed upon GP kinetics using SAS MODEL and the Gompertz function.

Results: Native FP contents of starch, CP and TP were 530, 199 and 184 g/kg DM. PS and PIP were 77 and 5 % of CP. FP silages had pH 4.6 and concentrations of lactic acid, ethanoic acid and ethanol of 18.8, 1.7 and 2.6 g/kg DM. They were aerobically stable for at least 7 d. *In vitro* GP dynamics were marginally affected by ensiling and ensiling + toasting. Starch granules and matrix structures were visually altered after toasting at 180 and 200 °C. In native FP, effective PRCP ranged from 94 (*K*p2) to 182 (*K*p12) and from 104 (*K*p2) to 178 g/kg DM (*K*p12) in experiment A and B, respectively. It was less affected by the treatments. Ensiling + toasting decreased the protein fraction B1, but increased B2 and B3 fractions, indicating decreased PS with increasing temperature (from 77 to 36 % of CP) or time (from 77 to 58 % of CP). In native FP, effective RUP ranged from 26 (*K*p2) to 34 or 35 g/kg DM (*K*p12) in both experiments. It was increased by ~ 10 g/kg DM after ensiling and further through toasting by finally ~ 20–60 g/kg DM ($P < 0.05$). PIP and the C fraction remained unaffected.

Conclusion: The *in vitro* tests have indicated that FP starch and protein can be protected in the rumen by combined ensiling and toasting, without the risk for protein damage. Attainable effects and benefits of silage toasting are, however, strongly dependent from temperature and exposure time.

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This study was supported by the German Federal Office for Agriculture and Food (BLE).

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91.

Testing effects of grinding, soaking and fermentation of different cereals on the 'structure' of the diet and stomach health in pigs

Zum Einfluss des Vermahlens und Einweichens sowie der Fermentation verschiedener Getreidearten auf die „Struktur" im Mischfutter sowie die Magengesundheit bei Schweinen

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Introduction: To achieve high digestibility rates, low particle size of feed is commonly recommended in feeding pigs. Additionally there is a relatively new feeding system which promises higher P digestibility and favored gut health[1].Finely ground feed is known to cause gastric lesions in pigs´ stomach. By feeding diets in liquid or in dry form no differences in stomach health were observed, when diets´ physical forms were the same[2]. The aim of this study was to evaluate effects of soaking and feed fermentation on the particle size and on stomach health in pigs. Furthermore it should be tested whether adding rolled cereals could prevent the development of severe gastric lesions.

Methods: Three different barley, wheat and rye varieties were tested (for each grain type: n=3). All cereals were ground in a hammer mill with a 3mm screen. This was followed by one dry (ds) and two wet sieve (ws) analyses [1h vs. 24h soaking time (st)]. For this purpose, sieve towers were used consisting of eight sieves (mesh sizes (mm): 3.15, 2.0, 1.4, 1.0, 0.8, 0.56, 0.4 and 0.2). In a second part of the study, fattening pigs were fed a diet consisting (in % of DM) of 48.8 rye, 29.3 rapeseed meal, 9.80 wheat, 9.80 barley and 2.46 mineral feed ad libitum for four weeks. The first group (n=5) was offered a liquid diet (particles>2.0mm: 7.30% / particles<0.2 mm: 32.8%). Group two (n=5) was fed the same diet, after 24h of fermentation with a starter culture (Schaumalac feed protect XP G; particles>2.0mm: 1.60% / particles<0.2mm: 65.5%). Finally, the other five animals were given a diet consisting of 60% fermented and 40% non-fermented feeds (particles>2.0mm: 25.5% / particles<0.2mm: 41.9%). The non-fermented cereals were ground by a roller mill only to increase particle size in the diet. Using a gastric score (0 = no changes, 1 = sligh-, 2 = moderat-, 3 = high hyperkeratosis, 4 = erosion, 5 = ulcer) the health of pars nonglandularis was assessed. The statistical evaluation was done by SAS® (sieve analyses: t-test / stomach examination: Wilcoxon test).

Results: For all cereals, a dependency of GMD on soaking was given. Above all, compared to dry sieve analysis wheat and rye meal were significantly ($p<0.05$) finer after a soaking period of 24h [wheat: 521±21.6µm (ds) → 524±45.0µm (ws, 1h st) → 305±67.6µm (ws, 24h st) / rye: 616±23.7µm (ds) → 487±35.7µm (ws, 1h st) → 250±14.8µm (ws, 24h st)]. In addition wheat and rye meal were also finer compared to all barley varieties [676±85.0µm (ds) → 713±77.6µm (ws, 1h st) → 586±90.4µm (ws, 24h st)]. Thus barley had a coarser structure than the other two cereals at any time. Rye meal had a coarser structure in dry sieve analysis than wheat meal. But in the wet sieve analysis wheat meal was coarser than rye meal. The feeding experiments yielded a stomach score of 2.70±1.30 for liquid offered feed. Thus, these stomachs were significantly ($p<0,05$) less affected than those of pigs fed the fermented feed (stomach score 5.00±0.000). Group three, which received the partly fermented diet had the lowest stomach score (1.40±0.652).

Conclusion: Soaking and fermentation had marked effects on the outcome of the sieve analysis (the strength of these observed effects depends on grain type: rye → low particle sizes). Changes in the physical form of diets due to grinding, soaking and fermentation led to consequences for gastric health in pigs. Within this study it could be confirmed that a loss of coarse particles in the feed is negatively correlated to stomach health. Furthermore stomach health was negatively influenced by fermented feed, but it has to be underlined that by adding coarse (structured) feed materials from roller mill (particles>2.0mm nearly 16 times higher in the partly fermented diet compared to the completely fermented diet) the positive effects of fermented liquid feeds can be used (shown by BUNTE 2018[3]) without inducing clinically relevant lesions like erosions or ulcerations in the pigs' stomach.

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92.

Variability of the equine intestinal microbiota after feeding of Jerusalem Artichoke meal

Variabilität der intestinalen Mikrobiota des Pferdes nach der Fütterung von Topinamburmehl

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Jerusalem artichoke (*Helianthus tuberosus*) meal (JAM) is used in horses to supply inulin-type fructans for prebiotic purposes. According to a meta-analysis, the effective dose of prebiotic active substances such as fructo-oligosaccharides (FOS) and inulin in horses is 0.2 g/kg body weight (bwt) and day (1). This includes the circumstance that fructans start to be decomposed already in the equine foregut (2) and particularly the stomach. Due to an increased microbial fermentation with higher production of short chain fatty acids (SCFA), the risk for gastric ulcers might increase (3).The aim was to determine the variability of the bacterial composition along the gastrointestinal tract (GIT) of horses following feeding of FOS and inulin from JAM in a dose close to the prebiotic one. We hypothesized, that this had an impact on the bacterial composition in the entire GIT including the stomach and small intestine.

Methods: During 3 weeks, 2 x 6 adult warmblood horses (534 ± 65 kg bwt; 14 ± 7.5 years old) received in 2 equal meals/d (crushed oat grains: 1.2 g starch/kg bwt d^{-1}; meadow hay: 1.5 kg/100 kg bwt d^{-1}) either JAM (0.15 g/kg bwt d^{-1}) or maize cob meal without grains as placebo (CON). On d21, the horses were euthanized ~ 1 h after the morning meal. Immediately post mortem, the digesta was collected from 7 different parts of the GIT (stomach: pars nonglandularis [PN], pars glandularis [PG]; small intestine [SI]; caecum [CAE]; colon: ventrale [CV], dorsale [CD] and transversum [CT]) and subsequently frozen. From the thawed material, DNA was extracted using a double-beat beating method combined with a commercial extraction kit (QIAmp DNA stool Mini Kit). Samples were amplified in a 2 step PCR using specific linker and barcoded primers for the 16S rRNA gene. Subsequently, samples were sequenced (GATC Biotech) using Illumina MiSeq sequencing and data analysed using QIIME software. Wilcoxon test was used to compare the relative abundance of species in the GIT segments and between the feeding groups. Diversity indices (Simpson 1-D, Shannon-Wiener, Simpson evenness) were compared in the implemented t-test in PROC mixed (SAS 9.4).

Results: Simpson 1-D and Shannon-Wiener diversity index indicated higher diversity in all GIT segments with JAM *vs* CON ($P < 0.05$). The relative abundance of species belonging to the phylum *Firmicutes* was highest (particularly in the stomach and small intestine) with JAM. Hereby, the genus *Lactobacillus* was predominant in the foregut with higher relative abundance with JAM *vs* CON ($P > 0.05$). In the hindgut, JAM caused a higher relative abundance of rare genera (≤ 2 %) and a lower relative abundance of the predominant genera (unclassified genus within the *Lachnospiraceae*).

Conclusion: Results suggest that the prebiotic supply *via* JAM interact with the autochthonous hindgut microbiota. Contrary to the declared target but in accordance with (2) and (3), feeding of JAM resulted in a higher relative abundance of the dominant bacteria (here: *Lactobacillus*) exclusively in the foregut whereby the impact on the hindgut was negligible. This indicates an only small prebiotic effect in the hindgut at the JAM dose supplied here. Higher contents of SCFA in the stomach might furthermore elevate the risk for gastric ulcers (3). Nevertheless, the JAM-induced higher bacterial diversity along the entire GIT, potentially contributing to an increased stability and resilience of the microbiota.

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This study was part of a DFG project.

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93.

Effect of rumen nitrogen balance and dietary protein source on intake, milk yield, nitrogen excretion, and behavior of dairy cows

Einfluss der ruminale Stickstoffbilanz und Proteinquelle auf Futteraufnahme, Milchleistung, Stickstoffausscheidung und das Verhalten in Milchkühen

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Lowering rumen-degradable protein intake may increase nitrogen (N) use efficiency (i.e., g milk N/g N intake) and reduce N emissions in dairy cattle. However, kinetics of feed N supply to rumen microbes and efficiency of microbial protein synthesis vary with diets. Hence, the aim was to study the effects of rumen nitrogen balance (RNB), dietary protein source, and their interaction on dry matter (DM) intake, milk yield, N excretion, and behavior of dairy cows.

Methods: Four diets were tested in 24 lactating Holstein-Friesian cows during 4 periods (12-d adaptation, 8-d sampling) in a complete Latin square design. Diets were fed for ad libitum intake and comprised of grass silage, maize silage, grass hay, second-cut grass hay, and barley straw as forages and four different concentrate mixtures in a forage to concentrate ratio of 55:45 (on DM basis). The concentrate mixtures mainly contained barley grain, sugar beet molasses, feed sugar, and either faba bean grains (FB) and RaPass® or SoyPass® (SP) and rapeseed cake, with FB and SP as two main protein sources (>35% of total dietary crude protein). Composition of concentrate mixtures were adjusted to create diets with RNB of 0 g N/kg DM (i.e., FB0, SP0) or -3.2 g N/kg DM (i.e., FB-, SP-). Both diets for each protein source were iso-energetic, but differed in N concentration. Individual feed intake was measured by weighing troughs and milk yield recorded with in-parlor milk meters. Samples of feed and milk were analyzed for their chemical compositions. Daily urine spot samples were analyzed for N, purine derivative (PD), and creatinine and fecal grab samples for N. Total fecal excretions were estimated using titanium dioxide as external marker. Apparent total tract digestibility (ATTD) of N was calculated. Chewing behavior was recorded in three cows per treatment) using pressure sensors (RumiWatch, Itin+Hoch GmbH, Liestal, Switzerland). The main effects of RNB, protein source, period, and their interactions were tested by PROC MIXED in SAS 9.4 using animal as random factor. Significance level was $P<0.05$.

Results: Mean daily DM intake (FB0 24.4 kg, FB- 23.5 kg, SP0 24.3 kg, SP- 24.2 kg/cow) and fat-energy-corrected milk yield (FB0 30.6 kg, FB- 28.6 kg, SP0 31.7 kg, SP- 31.3 kg/animal) were lower for FB- than FB0 ($P<0.01$ for both variables), but similar for both SP diets ($P\geq0.44$). Milk urea concentrations (FB0 238 mg, FB- 119 mg, SP0 282 mg, SP- 184 mg/kg) were lowest ($P<0.01$) and milk N use efficiencies highest (FB0 26.8%, FB- 32.0%, SP0 25.5%, SP- 29.1% of N intake) ($P<0.01$) for FB- and SP- diets, with greater differences observed between RNB levels for FB than for SP diets ($P<0.01$). The ratio between N and creatinine concentrations (g/l) in urine decreased with declining RNB from 12.3 to 6.7 for FB and from 13.8 to 9.5 for SP diets ($P<0.01$ for both protein sources), suggesting a low urinary N excretion in diets with negative RNB. The ratio between PD and creatinine concentrations (mmol/l) in urine were lower for FB- (3.9) than FB0 (3.6) ($P<0.01$), but similar for both SP diets ($P=0.40$), indicating a lower duodenal microbial crude protein flow for FB- diets. Fecal N excretion decreased when diets were changed from FB0 to FB- ($P=0.01$), but were similar for both SP diets ($P=0.22$). Yet, ATTD of N (g/100 g) was lower for FB- and SP- than FB0 and SP0, respectively ($P<0.01$ for both sources). Although eating time at the trough was lowest for FB- and SP- diets ($P<0.01$), rumination time was lower for FB- compared to other diets ($P<0.01$), possibly due to low fiber concentrations in FB- diets.

Conclusions: Reducing dietary RNB can improve N use efficiency and thus, decrease N emissions via urine. The effects of negative RNB on feed intake, performance, and digestibility in cows appear to be more pronounced in diets with rapidly degradable protein sources.

This work was financially supported by the Ministry of Science, Research and Arts of the State of Baden -Württemberg, Germany.

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94.

Differences in the *in vitro* rumen fermentation of tropical legumes and grasses and temperate legumes, and their neutral detergent fraction

Unterschiede in der Pansenabbaubarkeit zwischen tropischen Leguminosen, tropischen Gräsern und temperierten Leguminosen und deren Neutrale Detergenzien Faser

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Feeding legume forages is proposed as means to increase the nutritional status of ruminants. They appear to have an advantage over grasses, mainly associated with an increased crude protein (CP) supply that promotes intake, microbial growth and organic matter fermentation. In tropical regions, legumes have not been adopted in the extent of e.g. Lucerne (*Medicago sativa*) in temperate regions. It is now clear that tropical legumes do not share all positive attributes of their temperate counterparts, with decreased intake and digestibility appearing when grasses are substituted by legumes under conditions of similar dietary CP level (1). A high fiber content and a high lignification of tropical legumes may be at the helm of that contrast. Thus, the aim of this study was to compare the *in vitro* fermentation of tropical legumes, tropical grasses and Lucerne under conditions of similar CP, as well as the fermentation of their neutral detergent fiber (NDF).

Methods: Two tropical legumes, *Arachis pintoi* (Low NDF), *Stylosanthes guianenesis* (High NDF); two tropical grasses, *Pennisetum purpureum* (Low NDF), *Andropogon gayanus* (High NDF); and two Lucerne samples (21 and 35 days after emergence) were evaluated with the Hohenheim gas test (200 mg sample, 30 mL buffered rumen fluid) for 24 h in triplicates (2). The CP and NDF content of the forages (in g/kg DM) was: Arachis (158 and 380), Stylosanthes (158 and 524), Pennisetum (117 and 574), Andropogon (61 and 736), Lucerne_21 (303 and 166) and Lucerne_35 (228 and 301) for CP and NDF, respectively.

Four trials were conducted: Trial_1) Forages incubated alone; Trial_2) Forages incubated in combination with starch (70:30) and urea to ensure similar CP concentration in the medium; Trial_3) extracted NDF fractions from each forage were incubated alone; Trial_4) NDF fractions from tropical legumes and grasses were incubated in combination in proportions of 67:33 and 33:67. Gas production (GP, ml/200 mg) was measured as an indicator of substrate fermentability. Data were analysed using the GLM procedure of SAS software.

Results: In Trial_1 clear differences appeared in GP between the samples with the following order: Lucerne_21 > Lucerne_35 > Arachis > Stylo > Andropogon > Pennisetum, in line with expectations from their NDF and CP content, except for the grasses. When incubated under conditions of similar CP and with additional starch (Trial_2), GP was highest for Lucerne_21, but differences disappeared between Lucerne_35 and Arachis; differences between both grasses also disappeared. Both tropical legumes were still better than the grasses. In Trial_3, NDF from Lucerne had highest GP, but the NDF from tropical legumes was lower than both tropical grasses, indicating a lower fermentability of the NDF fraction from legumes. When combining NDF from legumes and grasses (Trial_4), the mixture of legumes with Pennisetum (low NDF) yield GP similar to that of the weighted average GP from the solely incubated samples; however, when legumes were mixed with Andropogon (High NDF) GP from the mixtures were higher than the weighted average from the solely incubated samples, highlighting an interaction effect between legumes and grasses when a low quality grass is substituted.

Conclusions: Based on the NDF and CP content, tropical legumes cannot compare with Lucerne, but under conditions of similar CP content and additional fermentable carbohydrates a good quality tropical legume shows a similar fermentation with a Lucerne forage. Tropical legumes appear to have an advantage over tropical grasses even when incubated under conditions of similar CP content with possible positive consequences when feeding legumes. Interestingly, the NDF from tropical legumes were less fermentable than that of grasses, meaning that legumes feeding can be detrimental if included at a high level and/or if legumes have a high NDF content. Synergistic effects appeared between legumes and grasses when the substituting a poor quality grass.

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95.

Heat stress and hyperosmolarity impact rumen bacteria and fermentation *in vitro*

Hitzestress und Hyperosmolarität beeinflussen die Pansenmikrobiota und – Fermentation in vitro

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In order to understand the factors involved in ruminal dysbiosis, it is important to assess the impact of the external factors under controlled conditions. The rumen microbes are continually challenged with variation in osmotic pressure, rumen temperature, and ruminal pH due to eating, drinking environmental temperature and dietary changes. Changes in microbiota due to heat stress, feed intake, and pH can influence the fermentation pattern in the rumen resulting in variation in digestibility, short-chain fatty acids (SCFA) production, and also methane emission. However, *in vivo* there are too many confounding factors, such as dry matter intake and rumen absorption. Therefore, this study investigated the effects of heat stress and hyperosmolarity on *in vitro* rumen bacteria and archaea and their associated fermentation.

Methods: Using an *in vitro* rumen simulation system (RSS), fermenters were assigned either to the control temperature of 39.5°C or 42°C to trigger heat stressed rumen conditions. Fermenters of both temperatures were fed either a control 50:50 forage to concentrate diet and supplied a standard McDougall's buffer (target normal pH = 6.6 and osmolarity = 295 mosmol/L) or fed a 35:65 forage to concentrate diet and supplied a diluted buffer (target pH = 6.0 and hyperosmolarity = 420 mosmol/L). Rumen fermentation was assessed using SCFA, gas production, and methane as markers. To identify alterations in the microbial community, samples were both sequenced for 16S rRNA gene and analyzed for archaea using quantitative PCR. Samples were analyzed with the Proc Mixed procedure of SAS with osmolarity, temperature, and the interaction as fixed effects, fermenter as a random effect and run as a repeated measure.

Results: With increased osmolarity, total gas production($P < 0.001$), CO_2 ($P = 0.001$), and CH_4 release ($P < 0.001$), as well as the percentage of isobutyrate ($P = 0.02$) all decreased. Hyperosmolarity also tended to reduce the total SCFA production (mmol/L; $P = 0.06$). Heat stress had only the tendency towards increasing the percent of valerate produced in the fermentation system ($P = 0.07$). Statistical analysis of the sequence data showed that hyperosmolarity negatively impacted rumen bacterial diversity as assessed by Chao1, Shannon and Simpson's indices, as well as decreased the total number of observed species. At the phyla level, *Bacteroidetes* tended to be increased under high osmotic pressure ($P = 0.10$), whereas *Chloroflexi*, *Elusimicrobia*, *Fibrobacteres*, *Lentisphaere*, *Plantomycetes*, *Spirochaetes*, *Tenericutes*, TM7, *Verrucomicrobia* and WPS-2 were significantly decreased under high osmotic pressure. *Chloroflexi* ($P = 0.04$) and *Euryarchaeota* ($P = 0.004$) phyla were increased under heat stress conditions. Archaea analysis using qPCR confirmed the increase in relative abundance under heat stress conditions however, the only archaea genera determined through sequence analysis, *Methanobrevibacter*, only showed a trend towards being increased in heat stress temperatures. Of the 116 genera analyzed, 5 were significantly impacted by temperature, 33 significantly impacted by osmolality and 4 exclusively impacted by the interaction between osmolarity and temperature. Correlation of significant genera with rumen fermentation parameters showed that the majority of those genera which decreased significantly with increased osmolarity were strongly negatively correlated to ruminal pH but not to osmotic pressure.

Conclusions: Temperature and osmolarity play important roles in rumen microbial function and consequently impact rumen fermentation. In our study, the effect of osmolarity was more pronounced compared to temperature. Current methods of sequencing do not have sufficient clarity to describe the role of Archaea.

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96.

Comparison of *in situ* ruminal crude protein and starch degradation values of single feeds and compound feeds for dairy cows produced thereof

Vergleich von in situ bestimmten Werten zum effektiven Rohprotein- und Stärkeabbau von Einzelkomponenten und aus diesen hergestellten Milchleistungsfuttern

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In situ studies are often conducted using single feed ingredients. However, compound feeds are widely used in feeding dairy cows. Thus, values for ruminal crude protein (CP) and starch (ST) degradation of compound feeds are calculated assuming interactions between single feeds used in the mix do not exist and values are additive. This study investigated whether the assumption of additivity of CP and ST degradation values of single feeds in compound feed is correct.

Methods: Twelve commonly used single feeds (maize, wheat, wheat bran, barley, soybean meal, soybeans, sunflower meal, faba beans, rapeseed meal, dried distillers' grains with solubles, maize gluten, and sugar beet pulp) were used in different proportions for production of 8 compound feeds targeting CP concentrations of 16, 18, 20, 22, 24, 26, 28, and 30 % of DM (named compound feed 1 to 8). Mixing was in a commercial feed mill (RKW Kehl, Germany) using standard industrial conditions without pelleting. All 20 samples were ground (2 mm sieve size), weighed into polyester bags (pore size 50 µm, bag size 10 × 20 cm), and incubated over 2, 4, 6, 8, 16, 24, 48 and 72 h in three ruminally fistulated lactating cows. An exponential model (1) was fitted to the degradation data and effective degradation (ED) of CP and ST for ruminal passage rates of 5%/h and 8%/h (ED5 and ED8) was computed. The EDCP and EDST of compound feeds were additionally calculated under the assumption of additivity by weighting observed values of single feeds for their individual CP or ST contribution to total CP or ST content of each compound feed. The ED values calculated this way for compound feeds were compared with the observed values using PROC MIXED of SAS 9.4 software. The model contained the way ED values were obtained (observed vs. calculated), the compound feed (1-8), and their interactions as fixed effects, while animal was considered as a random effect. Differences were deemed significant at the α level of 0.05.

Results: Observed ED5CP and ED8CP values for compound feeds ranged between 75 and 88 %, and 67 and 84 %, respectively. Calculated ED5CP and ED8CP values of compound feeds ranged between 77 and 87 %, and 70 and 83 %, respectively. A significant interaction between the way ED values were obtained and the compound feed was found for both, ED5CP and ED8CP. There was no significant difference between the way of ED values were obtained for most of the compound feeds, but calculated values were significantly higher in compound feed 4 (+4 percentage points) and 5 (+3 percentage points) for ED5, while calculated values for ED8 were significantly lower in compound feed 2 (-3 percentage points), and significantly higher in compound feed 4 (+5 percentage points) and 5 (+3 percentage points). Overall, differences between calculated and observed EDCP values did not exceed 5 percentage points. Observed ED5ST and ED8ST values for compound feeds ranged between 84 and 99 %, and 78 and 99 %, respectively. Calculated ED5ST and ED8ST values for compound feeds ranged from 84 to 97 %, and from 79 to 96 %, respectively. No interaction between the way ED values were obtained and the compound feed was found for EDST values. Overall, calculated EDST values were slightly but significantly lower than observed values (1 percentage point).

Conclusions: Although results showed some significant differences between calculated and observed ED values, differences were overall small and suggest that ED values of single feeds can be used to calculate ED values of compound feeds. Significant differences could not be related to any specific single feed. However, the significant differences showed that interactions between single feeds can exist when they are mixed, and the reasons for interactions need to be investigated.

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97.

Effects of different concentrate levels and buffer compositions on the induction of a subacute acidosis by applying the Rumen Simulation Technique

Einfluss von unterschiedlichen Kraftfuttermengen und Pufferzusammensetzungen auf die Etablierung einer subakuten Pansenazidose im RUSITEC

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Subacute rumen acidosis (SARA) is a common disorder in cattle, especially in high yielding dairy cows. The concentrate-rich diets, which are needed to meet the energy demands for milk production, provide a great amount of easily fermentable carbohydrates, leading to an accumulation of short-chain fatty acids and a decrease in rumen pH. When the acid production exceeds the physiological buffer mechanisms in the rumen, pH declines further and falls below critical thresholds. In literature, however, SARA is not clearly defined. Thresholds are pH < 5.8 (1) or 5.6 (2), while lactate accumulation remains low. The aim of this study was firstly to induce SARA in the RUSITEC model and secondly to observe the effects of different ratios of concentrate/roughage and the effects of the type of buffer on rumen fermentation parameters.

Methods: To establish SARA in an in vitro model the rumen simulation technique was applied (3) and acidotic conditions were induced in 6 out of 8 fermentation vessels. Four experimental runs were performed. Each experiment consisted of an equilibration period (EP), followed by a control period (CP I), an acidosis period (AP) and ended with a second control period (CP II). Acidosis challenge was provoked with two modified acidosis buffers. The buffer capacity was reduced by lowering the concentrations of bicarbonate and phosphate, compared to the standard buffer. Additionally, we compared three feeding models, which differed in the concentrate-to-roughage ratio (30:70, 70:30, changing ratio). Model-residuals were checked for normal distribution using Shapiro Wilk test. Effects of 3 periods, 5 time points and 8 combinations of rations with buffers to the feature parameters were analyzed using a three-way-ANOVA for independent and repeated measurements with post-hoc Tukey test for pairwise comparisons.

Results: During AP, the pH decreased to a constant value below the thresholds of pH 5.8 and 5.6, while lactate concentration never exceeded 1.0 mmol/l. The production of total short-chain fatty acids declined 3 to 4 days after acidosis induction, and the pattern of individual acids was altered. The proportion of acetate decreased ($p < 0.001$ day 5 of AP) and propionate proportion remained invariant, while butyrate proportion increased (at least $p < 0.05$ day 5 of AP). Differences among buffer treatments were detectable. Effects of the diet were measured for acetate production, as initially, groups with 70% of concentrate exhibited a higher production, compared to low concentrate groups (at least $p = 0.0445$). Groups fed a high concentrate diet possessed a higher NH_3-N concentration in CP I, compared to groups with a low concentrate ration. However, most fermentation parameters recovered, when standard buffer was reinfused.

Conclusion: The present study provides an in vitro SARA model, which might be used to pretest acidosis prevention strategies prior to performing animal experiments. Furthermore, this experiment suggested a higher impact of buffer composition rather than the concentrate/roughage ratio on fermentation parameters, such as pH, lactate production and SCFA pattern.

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98.

Fermentation characteristics of feeds with different carbohydrate composition incubated at low and high dilution rate in the RUSITEC

Fermentationscharakterisika von Futtermitteln mit unterschiedlicher Kohlenhydratzusammensetzung inkubiert mit niedriger und hoher Verdünnungsrate im RUSITEC

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Introduction: Various factors have the potential to influence ruminal fermentation characteristics like microbial protein formation. This study investigated how feedstuffs rich in different easily fermentable carbohydrates and the dilution rate influences the fermentation characteristics in the RUSITEC system.
Material and methods: Three different carbohydrate-rich feeds (barley / starch; molassed beet pulp / sugar, pectin, (hemi)cellulose; soy hulls / cellulose) and two different buffer dilution rates (low and high, 1.5 and 3.0 % of the fermenter volume/h, respectively) were investigated (n=6 for each combination). Buffer solution was prepared according to McDougall (1) and modified to contain 0.53 and 0.26 g NH_4Cl/l for low and high dilution rate, respectively. Amount of infused salts per day were the same for both rates. Each run consisted of eight days of adaptation and four days of sampling. The diet contained 5 g hay, 2 g rapeseed meal and 4 g of the carbohydrate rich feed on dry matter basis with 154, 151 and 177 g CP/kg DM, 380, 434 and 528 g aNDFom/kg DM and 200, 239 and 331 g ADFom/kg DM for diets containing barley, beet pulp and soy hulls respectively. Formed microbial mass and short chain fatty acid (SCFA) content were measured every sampling day in the effluent, as well as methane (CH_4) content in the gas volume. For microbial mass determination an aliquot of 40 ml effluent was centrifuged (500 x *g*, 10 min, 4°C), the supernatant was centrifuged again (20,000 x *g*, 30 min, 4°C). The SCFA analysis was carried out by gas chromatography. Total gas volume was measured using a water displacement apparatus. Methane content was analysed using an infrared analyser. For statistical analysis the mixed procedure of SAS (version 9.4) was used with feed, dilution rate and their interaction as fixed and RUSITEC run as random effect. Sampling day way considered as repeated measure. Least square means were compared by Tukey-Kramer test.
Results: Microbial biomass recovered in the effluent was higher ($p<0.001$) for fermenters with high dilution rate (302 and 433 mg/d for low and high dilution rate, respectively), while the different feeds had no impact. The amount of CH_4 was lower ($p<0.001$) for fermenters with high (58 mmol/d) compared to low dilution rate (35 mmol/d). Substrate source had no effect on the CH_4 amount. The total amount of SCFA produced in the fermenters with high dilution rate was lower ($p<0.001$) than in the fermenters with low dilution rate (49 vs. 54 mmol/d). The feeds had here a weak significant effect ($p=0.047$) with a higher value for barley (53 mmol/d) than for soy hulls (50 mmol/d) and beet pulp (51 mmol/d) in between not differing from the other. Feed and dilution rate had a significant effect on acetate content with higher values for soy hulls and beet pulp (29.7 and 28.4 mmol/d, respectively) than for barley (25.9 mmol/d). The propionate content in the effluent of barley and beet pulp fermenters (both 12.7 mmol/d) was higher than in fermenters with soy hulls (11.9) ($p=0.006$), also dilution rate ($p=0.048$) and its interaction with feed had an effect ($p=0.005$). The acetate:propionate ratio was higher for soy hulls (2.52) than beet pulp (2.26) than barley (2.04) ($p<0.001$) and higher for the high dilution rate ($p>0.001$), the interaction was significant as well ($p=0.012$). The amount of butyrate there was an effect of dilution rate, feed and their interaction detected with higher values for barley than beet pulp than soy hulls.
Conclusions: The dilution rate had greater impact on important fermentation characteristics like formation of microbial mass, total SCFA and CH_4 production than carbohydrate source. Our results support that increased dilution rate could be beneficial for a more efficient formation of microbial mass.

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99.

Betaine as a rumen fermentation modulator during physiological, hyperthermal and hyperosmotic rumen conditions *in vitro*

Untersuchungen zum Einfluss von Betain als Fermentationsmodulator unter physiologischen Pansenbedingung und unter dem Einfluss einer höheren Temperatur bzw. einer Hyperosmolarität in vitro

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Betaine or trimethylglycine is found in microorganisms, animals and plants, where it is abundant e.g. in sugar beet and wheat. These plants are parts of cattle diets and betaine may have contributed to their positive effects on rumen fermentation and animal performance (1), yet direct effects of betaine on rumen fermentation are not well studied. Betaine is a potent methyl donor and compatible osmolyte and therefore can contribute to microbial metabolism as well as offer protection against stressors such as hyperosmolarity and high temperature. Betaine is also catabolized by microbes yielding trimethylamine and acetic acid. Altogether, we hypothesized that betaine promotes rumen fermentation and desirably modulates rumen fermentation especially during hyperthermal and hyperosmotic rumen conditions.

Methods: The experiment was conducted using a rumen simulation technique with 12 fermenters in total per run. The inoculum was obtained from 3 donor cows (non-lactating Holstein). The experimental plan was a 2 × 2 × 3 factorial arrangement in a randomized block design which was conducted in 6 experimental runs (n = 6 per individual treatment). Three different doses of betaine (Actibeet® L, 40% betaine, obtained from sugar beet molasses) were used: 0% (control), 0.30% of diet DM (low), and 1.67% of diet DM (high), each was assigned to 2 incubation temperatures: normal rumen temperature at 39.5 °C and 42 °C representing a hyperthermal condition and 2 osmotic conditions including normal (target osmolality at 295 mOsmol/kg and pH of 6.6) and hyperosmotic (target osmolality at 420 mOsmol/kg and pH of 6.0). The osmolarity and pH of the incubation fluid for normal and hyperosmotic conditions were successfully established via diet (50 and 65% concentrate, respectively) and buffer (McDougall's buffer and diluted buffer plus NaCl, respectively). Each experimental run lasted 10 d and the last 5 d served as sampling period to determine fermentation characteristics. All data analyses were done using the MIXED procedure of SAS. Daily data within fermenter were analyzed as a repeated measure and the main effects included betaine dose, temperature, osmolarity, and their interactions. The experimental run was considered as the random effect.

Results: Addition of betaine did not affect pH and redox but high betaine increased osmolarity (+9 mOsmol/kg, $P < 0.05$). Independent of incubation conditions, on average, both betaine doses increased the concentration of short-chain fatty acids (SCFA) (110 vs. 104 mmol/L), high dose increased fermentation gas (516 vs. 417 ml/d) and ammonia (14 vs. 11 mmol/L) compared to control ($P < 0.05$) and methane formation was linearly increased with dosage ($P < 0.01$). The hyperthermal stress suppressed SCFA production but this was counteracted by the addition of betaine as shown by the highest SCFA concentration with the high betaine incubated at 42 °C. Hyperosmolarity decreased fermentation as shown by lower gas production and SCFA formation. With betaine, slight improvements were found but did not reach significance. The SCFA profile was mainly affected by an interaction between betaine and osmolarity. With normal osmolarity, betaine increased the proportion of acetate at the expense of propionate whereas betaine promoted the proportion of propionate under the hyperosmotic condition.

Conclusions: Hyperthermal and hyperosmotic stress suppressed ruminal fermentation. Addition of betaine promoted ruminal fermentation even under the hyperthermal condition. Betaine acted as an acetogenic compound under physiological rumen conditions but can shift SCFA to propionate, suggesting its glucogenic effect in cattle, under hyperosmotic conditions. The increase in methane and ammonia indicates the catabolism of betaine by rumen microbes.

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This study was supported by AGRANA Stärke GmbH, Vienna, Austria.

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100.

Effects of yeast supplementation on performance parameters and rumen fermentation profile in dairy cows

Effekte einer Supplementierung mit Hefe auf Leistungsparameter und Pansenfermentation von Milchkühen

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The aim of this study was to investigate the response to an autolyzed yeast product (AY, Levabon® Rumen E, Biomin Holding GmbH, Austria) on milk yield, feed efficiency and rumen fermentation profile in dairy cows.
Methods: The study was conducted at the research facility Blanca from the Pyrenees, Spain. A total of 63 lactating multiparous Holstein cows (body weight (BW): 617±80.0 kg, days in milk (DIM): 127±26.3, milk yield: 32.7±6.95 kg/d at the beginning of the study) were randomly allocated to two different treatment groups. Groups were supplemented with either 0 (CTRL) or 20 g/cow/day of AY, which was incorporated into the concentrate of a total mixed ration (TMR). The TMR was based on (% of DM) barley (20.8), corn (18.0), ryegrass (15.5) and fescue hay (14.5) and offered in electronic feed bins, controlling access of cows to allocated dietary treatments and allowing individual application of treatments and recording of feed intake. The study duration was eight weeks, including two weeks of adaptation. Cows were milked twice daily and individual milk yield was recorded using electronic milk meters. Individual dry matter intake (DMI) was recorded using electronic feed bins. Rumen fluid from 15 cows per treatment was sampled at days 14, 42 and 56 of study and analyzed for pH and volatile fatty acid concentration (d 14, d 42 and d 56 for pH and d 14 and d 56 for VFA). Data were analyzed using a mixed-effects model including the fixed effects of treatment (CTRL or AY), time of sampling and their 2-way interaction, as well as the random effect of cow.
Results: Feed efficiency was calculated as milk yield (kg/d) over DMI (kg/d). DMI ($P < 0.05$) decreased and milk yield increased ($P < 0.05$) in AY supplemented cows as the time of exposure to treatment increased compared with CTRL cows. As result, cows fed AY had greater feed efficiency ($P < 0.001$). Rumen pH tended ($P = 0.06$) to decrease and rumen molar proportions of propionate increased ($P < 0.05$) in AY cows compared with CTRL cows at 56 d of study.
Conclusion: From this study, we can conclude that supplementation of AY may modulate fermentation of dry matter in the rumen, and in turn improve milk production and feed efficiency.

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101.

In vitro disappearance of betaine by ruminal microbes under physiological, thermal-stressed and osmotic-stressed rumen conditions

In Vitro Abbau des Betains von Pansenmikroben unter physiologischen und thermisch-osmotischen Stressbedingungen

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Heat and osmotic stresses jeopardize the performance of livestock through their negative impacts on rumen microbial fermentation. Dietary manipulation with betaine is an option to counteract such stresses because betaine is a not only a direct substrate for rumen microbes but also a compatible osmolyte, methyl donor, and molecular chaperone (1). We hypothesized that betaine is rapidly utilized by microbes for their protection against hyperosmotic stress as well as thermal stress. Investigating ruminal degradation kinetics of betaine is the first important step to understand and predict the effectiveness of betaine as a rumen modulator. The present study investigated the degradation kinetics of betaine under physiological, thermal-stressed and osmotic-stressed rumen conditions.

Methods: The experiment was conducted using a rumen simulation technique with a total of 12 fermentors. The test conditions were arranged as a 2 × 2 × 3 factorial design including 2 temperatures of normal (39.5 °C) and thermal stress (42 °C), two osmotic conditions: physiological (~295 mOsmol/kg and pH of 6.6) and hyperosmotic (~420 mOsmol/kg and pH of 6.0), and 3 betaine concentrations in the incubation liquid at 0 (Control), 51 (LB) and 286 (HB) ppm. The target pH and osmolarity were successfully achieved by means of buffer and diet. Fermenters with normal pH and osmolarity were infused with McDougall's buffer and fed a 50% concentrate diet, whereas those with low pH and hyperosmolarity were treated with a 65% concentrate diet and diluted buffer plus NaCl. The trial consisted of 6 runs resulting in n = 6 per individual treatment. Each run consisted of 10 d with the last 5 d used as sampling period. On d7, the incubation fluid was taken at 0, 1, 2, 4, 6 and 24 h after betaine dosing for betaine analysis using HPLC. Incubated feed bags from the last 5 d were used for determination of nutrient disappearance. The betaine disappearance kinetics was fitted as $y=ae^{bx}$ using the NLIN procedure of SAS. The resulting kinetic parameters as well as nutrient disappearance data were statistically analyzed for the 3 fixed factors and their interactions using the MIXED procedure of SAS.

Results: There was only a trend toward betaine × osmotic condition ($P < 0.10$) for crude protein and fat disappearance which were decreased by betaine under hyperosmotic stress conditions but not during normal conditions. The analyzed betaine concentration at 0 h was 56 ± 6 and 314 ± 14 ppm (mean ± SD) for LB and HB, respectively. In general, betaine rapidly disappeared within the first 6 h of incubation and no betaine could be detected at 24 h. The disappearance was affected by osmolarity of the incubation fluid, but not by thermal stress, and this was dose-dependent. Accordingly, the rate (b) of betaine disappearance was highest for LB incubated under the physiological rumen condition (- 0.80 and -0.90 for normal and thermal stress, respectively), but HB led to a slower disappearance rate (-0.50 in both incubation temperatures) ($P < 0.05$). Under hyperosmotic stress, both LB and HB displayed similarly low rates (b ranged from – 0.35 to -0.48). Shown as estimated remaining concentration of betaine, the physiological condition with LB was 44% at 1 h and 1% at 6 h after addition, while those of the other treatments ranged from 64-73% and 6-14% at 1 and 6 h, respectively ($P < 0.05$).

Conclusions: Nutrient degradation was minimally affected by betaine addition. Disappearance of betaine was rapid but it can be slowed down by the hyperosmotic stress. Data suggest that when given enough time rumen microbes are able to utilize large amounts of betaine possibly for their cell metabolism which may contribute to their fermentation activity.

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This study was supported by AGRANA Stärke GmbH, Vienna, Austria.

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102.

Determinating the precaecal digestibility of alfalfa *(Medicago sativa)* and red clover *(Trifolium pratense)* products in the organic feeding of broilers

Bestimmung der praecaecalen Verdaulichkeit von Luzerne- und Rotkleeprodukten (Medicago sativa, Trifolium pratense) in der ökologischen Broilerfütterung

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Based on the crude protein (XP) content and amino acid (AA) profile, alfalfa and red clover products offer high potential as a protein source in the organic broiler nutrition. The present study evaluates the XP and AA digestibility of different alfalfa and red clover products (dried alfalfa leaves (AL), alfalfa whole plant silage (AS), dried red clover leaves (RCL), red clover whole plant silage (RCS)).

Methods: A total of 468 male Hubbard-JA-757 broilers (29 days old) were distributed in 13 groups (Control (C), AL1-3, AS1-3, RCL1-3, RCS1-3) (36 animals per group) with 4 replicates. One replicate (= one pen) consisted of 9 animals. During the first 21 days (phase 1) an industrial organic starter was fed to all groups. Due to high crude fiber contents and the occurrence of antinutritive substances (saponins) in alfalfa and red clover plants, the experimental design differed from the usual methodology of digestibility determination. Phase 2 (day 22-28) served as adaptation to the different test feeds. Therefore, all diets, except C, contained 15% of the alfalfa or red clover products. For digestibility calculation the levels of test feeds in all diets were either de- or increased or remained the same in phase 3 (day 29-41/42), (AL/AS/RCL/RCS1: 10%; AL/AS/RCL/RCS2: 15%; AL/AS/RCL/RCS3: 20%). Diets of phase 3 contained free AA to ensure a constant feed intake between all groups and low effects of basal endogenous losses (1). The feed mixtures included corn starch as a replacement of the tested alfalfa and red clover products. Titanium dioxide was added as a non-absorbable marker for digestibility calculation. Weight gains, feed intake and losses were monitored during all 3 phases. Feed intake data collected during the period from day 30 to 41/42 served for digestibility calculation. On day 41/42 the broilers were asphyxiated with CO_2. The distal two thirds of the intestine section between Meckel's diverticulum up to 2 cm anterior to the ileocecal junction were flushed with distilled water. The content was pooled for all broilers of one pen, freeze-dried and analyzed for XP, AA and titanium. The precaecal digestibility (pcd) was calculated by regression (2). Statistical analysis of performance parameters was performed by SPSS 20.0 (2011), procedure GLM (Tukey test).

Results: After the introduction of test feeds in phase 2 the feeding groups differed significantly in live weights (LSM±SE in g; AS: 703.6 ± 6.9^{a}; C: 677.3 ± 8.4^{b}; RCL: 672.0 ± 6.9^{bc}; RCS: 652.7 ± 6.9^{bc}; AL: 648.5 ± 6.9^{c}). All feeding groups gained weight from phase 2 to phase 3. At the end of phase 3 animals fed AS showed the highest and broilers fed RCS the lowest live weights. The pcd of XP and AA for AS (Methionine: 70%, Lysine: 76%) and RCS (Methionine: 83%, Lysine: 67%) was higher than for AL (Methionine: 51%, Lysine: 39%) and RCL (Methionine: 58%; Lysine: 48%). Regarding the higher XP and lower crude fiber content of AL and RCL higher results were expected. Antinutritive substances like saponins, which have the potential to accumulate in leaves (3), may have influenced the digestibility.

Conclusions: Whole plant silages of alfalfa and red clover showed higher digestibilities for XP and AA than dried leave products of the same plants. This might be due to the accumulation of antinutritive saponins in leaves. For an establishment of alfalfa and red clover products as a reliable protein and crude fiber source in organic poultry nutrition, further investigation concerning saponin contents and their biological activity in the gastrointestinal tract are required.

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This study was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE).

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103.

Fermented wheat bran in diets for laying hens: Effect on nutrient digestibility/retention and microbiota

Fermentierte Weizenkleie im Legehennenfutter: Einfluss auf die Nährstoffverdaulichkeit/Retention und das Darmmikrobiom

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Due to an increasing competition between humans and livestock animals regarding cereal grains, the supplementation of feed with by-products such as wheat bran represents a sustainable and ecologically practicable approach. Fermentation can be considered as a dynamic process degrading dietary fiber and has commonly been used to improve the shelf-life and/or nutrient utilization of feed (1). In the course of the fermentation, the bioavailability of minerals can be elevated and the microbiological and physiological balance, as well as the gut health, can be ameliorated (2). Taking into account these aspects, this study was carried out to investigate the influence of fermented wheat bran on digestibility, retention and gut microbiota characteristics of laying hens.

Methods: Two fermentation strategies were applied. One batch was fermented using *Pleurotus eryngii* and the second was fermented using a combination of *Pleurotus eryngii* plus *Lactobacillus paracasei* (lac 034) and *Lactobacillus plantarum* (Lac 900). The subsequent feeding experiment was conducted using 24 laying hens. According to their body weight hens were randomly distributed to separate metabolism cages. Each treatment comprised six individuals: (I) a control treatment fed a common diet; (II) a treatment fed 15% native wheat bran; (III) a treatment fed 15% with *Pleurotus eryngii* fermented wheat bran; (IV): a treatment fed 15% with *Pleurotus eryngii*, *Lactobacillus paracasei* (lac 034) and *Lactobalillus plantarum* (Lac 900) fermented wheat bran. Subsequently, excreta from one hen were collected for six days, pooled to one sample and subjected to further analyses. Homogenized samples were dried and starch, neutral detergent fiber, gross energy and calcium, sodium, zinc and phosphorus were analyzed according to the methods of Naumann and Bassler, 2012 (3). After 21 days, all animals were slaughtered by a standardized procedure and intestinal chyme samples from jejunum, ileum and caecum were collected. The DNA was isolated using a PowerFecal® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA) and further sequenced by Illumina MiSeq v3 chemistry. All applied analyses were performed in duplicates, data were analyzed according to a two-way ANOVA using the MIXED procedure of SAS Enterprise Guide 7.1.. The Tukey Kramer test was applied for the multi comparisons of the means ($\alpha = 0.05$).

Results: The supplementation of 15% native and fermented wheat bran did neither affect the digestibility nor the retention of nutrients. The distinction of microbial composition in the jejunum and ileum was hardly pronounced. The phyla *Firmicutes*, *Actinobacteria* and *Proteobacteria* were most abundant in both segments, whereas *Bacteroides*, *Parcubacteria* and *Planctomyces* were only present in the jejunum in higher abundances. While the phylum *Firmicutes* was present in the jejunum and ileum at 64.6% and 91.4%, respectively, its abundance in the cecum (16.9%) was low. The most abundant phyla in the cecum were *Bacteroides* (65.5%), *Verrucomicrobia* (5.3%) and *Elusimicrobia* (4.8%). Nine genera in the cecum were evident: The relative abundance of *Bacteroides* was the highest (22.8%) in the hens fed with diet (IV) and the lowest (18.1%) in diet (I). In contrast, *Rikenellaceae* was most abundant (30.0%) in (I) and showed the lowest (23.6%) levels in (IV). *Lactobacillus* was the most abundant genera in the ileum and the jejunum. Regarding the different feeding strategies applied the presence of *Lactobacillus* in the ileum varied from 49.6% in diet (I) to 71.0% in diet (III) and in the jejunum from 48.8% in diet (VI) to 66.1% in diet (I).

Conclusion: Regardless of its status (fermented or not), a wheat bran-supplemented diet had no significant effect on digestibility and retention rates in laying hens. However, gut segment-specific differences were observed regarding microbial composition, whereas the differences between the feeding strategies within the gut segments were hardly pronounced.

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104.

Effect of resistant starch type 4 on intestinal passage rate, nutrient flow, microbial metabolites and gut microbiome composition in pigs

Einfluss einer transglycosidierten Stärke auf die Darmpassage, Nährstoffffluss, mikrobielle Metabolite und Zusammensetzung des Darmmikrobioms beim Schwein

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A large proportion of the observed health benefits of resistant starch (RS) are mediated via the gut microbiota and fermentation metabolites (1). Due to different bulking and rheological properties of RS, the intestinal availability of nutrients for microbial action is not only determined by the digestibility of the starch for host enzymes but a function of digestibility and intestinal rate of passage of digesta. In the present study, we aimed to investigate the effect of RS type 4 (RS4) on the ileal passage rate and quantified the ileal flow and hindgut disappearance of nutrients in growing pigs. In studying the bacterial microbiome and SCFA, we further assessed the dependencies between nutrient availability and alterations in bacterial composition and metabolite profiles in ileal digesta and feces.

Methods: Castrated male growing pigs (n=7; Large White, 55 kg, 4-5 months of age) fitted with an ileum T-cannula were randomly allotted to one of two diets in a complete crossover design with two 16-day periods. Each replicate period consisted of a 10-day adaptation to diets, followed by 3-day collection of feces and 3-day collection of ileal digesta. Cornstarch (72.1%)-casein (18%) based diets differed in the starch component, being rapidly digestible waxy maize starch in the CON diet, whereas in the RS4 diet 50% of the waxy maize starch was replaced by transglycosylated starch (Agrana Research and Innovation Center GmbH (ARIC), Tulln, Austria). Cromium-EDTA and ytterbium oxide as liquid and solid markers were mixed into the morning meal on day 16. Ileal and fecal DNA isolates were used for sequencing on a MiSeq Illumina Platform targeting the 16S rRNA gene (V3-V4 region). Sequencing data were analyzed using QIIME and 'DESeq2' and 'mixOmics' packages in R. Data of ileal transit, nutrient flow and short-chain fatty acids (SCFA) were subjected to ANOVA using PROC MIXED of SAS.

Results: RS4-fed pigs had a shorter retention of the solid phase marker in the stomach ($P=0.04$) and, as a trend ($P=0.06$), in the small intestine compared to CON-fed pigs. Also, TGS-fed pigs had a 2-fold higher ileal dry matter (DM) flow, being mainly the result of the 5-fold greater ileal starch flow compared to CON-fed pigs ($P<0.001$). Hindgut fermentation was enhanced in RS4-fed pigs as indicated by the 1.3- and 4.3-fold increase in DM and starch disappearance between ileum and feces and the 3-fold increase in ileal and fecal total SCFA compared to CON-fed pigs ($P<0.05$). The importance of the ileal and fecal DM and starch flow for ileal acetate and isobutyrate and fecal butyrate, respectively, was supported by relevance network analysis ($r>8$). Sparse-partial least squares discriminant analysis identified unclassified genera within *Desulfovibrionaceae*, *Dehalobacteriaceae* and *Ruminococcaceae* in ileal digesta as well as *Oscillopira*, *Megasphaera*, unclassified *Dehalobacteriaceae* and *Dialister* in feces as the most relevant bacteria as affected by the starch type. Relevance networks showed negative associations between ileal abundances of *Selenomonas*, *Bifidobacterium* and *Butyrivibrio* and gastric retention of digesta solids ($r>0.8$). In feces, especially *Megasphaera* benefited, whereas *Oscillospira* was depressed by the increase in DM and starch flow with the RS4-diet as indicated by the relevance network ($|r|>0.9$). Both *Acidaminococcus* and *Megasphaera* were further positively and *Oscillospira* negativey associated with acetate and caproate ($|r|>0.8$).

Conclusion: Results showed that, aside from effects due to the largely enhanced ileal starch flow, the RS4 modified bacterial abundances by decreasing the digesta retention in the upper digestive tract. Moreover, relevance networking identified the most influential bacterial genera in ileal digesta and feces that were directly affected by the change in starch type and benefited from the altered starch flow.

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105.

Digesta passage in nondomestic ruminants: separation mechanisms in 'moose-type' and 'cattle-type' ruminants, and seemingly atypical browser species

Digesta-Passage bei Wildwiederkäuern: Trennmechanismen bei ‚Elchtyp'- und ‚Rindertyp'-Wiederkäuern, und scheinbar atypischen Laubäsern

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Ruminants have been classified by their botanical diet as browsers, grazers or intermediate feeders, and by their digestive physiology as 'moose-type' or 'cattle-type' (1). 'Cattle-type' ruminants have stratified rumen contents and a clear difference in the mean retention time (MRT) of fluid vs. small particles in the reticulorumen (RR), with a high 'selectivity factor' (SF = $MRT_{particle}RR/MRT_{fluid}RR$, > 1.80), and are typically grazers and intermediate feeders. 'Moose-type' ruminants have unstratified rumen contents and lower SF (< 1.80), possibly because of defensive salivary proteins that lead to limited amounts of high-viscosity saliva, and are typically restricted to being browsers. How strict the correlation between physiology and diet is remains unknown, with several key ruminant species not studied to date. Here, we report previously unpublished results of 55 individual passage studies in 4 and 6 species that have and have not been investigated so far, respectively, to further contribute to testing the physiology-diet correlation.

Methods: We used Co-EDTA as a fluid and Cr-mordanted hay particles (<2mm) as particle markers, following a standard protocol (2), in captive individuals of various species between 2004 and 2018, either as part of other studies, or only for the measurement of MRTs. Previously studied species included cattle (*Bos taurus*, n=2, on grass hay), giraffe (*Giraffa camelopardalis*, n=3 on 3-5 diets each), moose (*Alces alces*, n=4 on 4-5 diets each and n=2 on a single diet), and waterbuck (*Kobus ellipsiprymnus*, n=5, zoo diets). Species not previously studied included nyala (*Tragelaphus angasii*, n=6, zoo diet), sitatunga (*T. spekii*, n=4, zoo diet), bongo (*T. eurycerus*, n=3, zoo diet), Arabian oryx (*Oryx leucoryx*, n=1, grass hay/lucerne), European bison (*Bison bonasus*, n=1, grass hay), and gerenuk (*Litocranius walleri*, n=1, zoo diet). Results are compared to the percentage of grass in the natural diet taken from the literature, as an indication whether species are mainly grazers or browsers.

Results: Moose (5% grass, SF 1.46 ±0.22) and giraffe (1% grass, SF 1.42 ±0.23) as classical 'moose-type', and cattle (70% grass, SF 2.04) as the classical 'cattle-type' ruminants yielded results similar to those previously published, as did waterbuck (84% grass, SF 2.46 ±0.49). These findings corroborate that the SF represents, to a large extent, a species-specific characteristic. Results in oryx (75% grass, SF 2.60) and sitatunga (68% grass, SF 1.81 ±0.21) correspond to the concept of 'cattle-type' ruminants with a high SF being grazers or intermediate feeders. However, European bison (10% grass, SF 2.74), nyala (20% grass, SF 1.95 ±0.25), bongo (13% grass, SF 2.39 ±0.54) and gerenuk (0% grass, SF 2.25) appear as 'cattle-type' ruminants with high SF yet a browse-dominated diet, which has not been reported so far.

Conclusion: While the results do not challenge the view that a 'moose-type' digestive physiology is an adaptation to browse diets, they indicate that it may not be the only adaptation that enables ruminants to use browse. Apparently, having a 'cattle-type' digestive physiology with a high SF does not necessarily prevent a browsing diet niche. High-SF browsers might have the benefit of an increased harvest of RR microbiota (3); how they defend themselves against secondary plant compounds in browse remains to be investigated.

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106.

Effects of abomasally infused starch and amylase on faecal excretion of microbial crude protein and short-chain fatty acids in heifers

Effekte abomasal infundierter Stärke und Amylase auf die fäkale Ausscheidung von mikrobiellem Rohprotein und kurzkettigen Fettsäuren bei Färsen

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Shifting starch digestion from the rumen to the small intestine is assumed to increase energy and glucose supply to lactating dairy cows, but previous experiments demonstrated limited starch digestion in the small intestine of ruminants. The hypothesis of the present study was that postruminal starch digestion is limited by pancreatic amylase activity when high amounts of starch (> 1 kg/d) escape ruminal fermentation. An exogenous amylase was abomasally infused to show improved small intestinal starch digestion by reduced faecal excretion of microbial crude protein (MCP) and short-chain fatty acids (SCFA) as end-products of hindgut fermentation.

Methods: Four rumen-fistulated heifers (~565 kg BW) were assigned in a cross-over trial with two experimental periods lasting 35 d each with 10 d of diet adaption followed by 25 d of sample collection. From day 6 to 10 d of each experimental period 724 g/d corn starch were abomasally infused for adaption to starch digestion. During the sampling period, starch was infused at five infusion levels (953, 1213, 1425, 1733 and 1993 g/d) each for a 5-d period. Starch was infused as suspension in 10 L water for 10 h/d with or without exogenous amylase. Latter was infused as solution in a dosage of 260.000 U/kg starch. The heifers were fed 5.5 kg/d of a diet targeted to contain no starch and consisting of 65% grass hay, 33% dried beet pulp, 0.9% urea and 1.2% of a mineral and vitamin premix (DM basis) in two equal meals per day. Titanium dioxide was ruminally administered (10 g/d) in two dosages per day for estimation of faecal excretion. Faecal grab samples were obtained each day during collection period and analysed for crude protein (CP), purine bases (PB) and SCFA. Microbial N excretion was estimated from PB assuming a purine N: microbial N ratio of 0.116 (1). MCP was calculated assuming a N content of 18%. Differences between treatments and infusion levels were analysed using the GLM procedure of SAS. Linear and quadratic orthogonal contrasts were tested for the effects of starch infusion level. Separate simple regression analysis (PROC REG; SAS) was used to determine the faecal excretion of microbial N as a function of the amount of starch infused.

Results: Faecal excretion of CP, MCP and SCFA increased linearly with increasing level of starch infusion ($P < 0.05$) averaging 289, 51 and 37 g/d at the lowest and 297, 59 and 55 g/d at the highest level of starch infusion, respectively. Microbial N excretion increased by 0.12 g/100 g starch infused ($P = 0.005$; R^2=0.19). However, none of these parameters were affected by amylase administration ($P > 0.05$).

Conclusion: Missing effects of amylase administration indicate that pancreatic amylase activity is not the primarily limiting factor of postruminal starch digestion in heifers when corn starch is abomasally infused in amounts up to 2 kg/d. Furthermore, the linear increase of MCP and SCFA excretion indicate rather a constant proportion of starch being praecaecally not absorbed than a limited quantity of starch which can be digested and absorbed in the small intestine. Assuming an efficiency of CP synthesis in the hindgut of 1 g N/100 g starch fermented (2), and an unaffected fibre fermentation in the hindgut (which needs further evaluation), disappearance of starch and end-products of starch hydrolysis from the small intestine averaged 88%.

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107.

Impact of 'controlled fermentation' of rye based compound liquid diets on extract viscosity (diet / digesta) and its relevance regarding digestibility in pigs

Einflüsse der Flüssigfutter-Fermentation eines roggenreichen Mischfutters auf die Extraktviskosität im Futter und Chymus sowie ihre Bedeutung für die Verdaulichkeit beim Schwein

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Introduction: Rye is characterized by its high contents of soluble arabinoxylans (rye: 30.9 g/kg DM compared to wheat: 13.9 g/kg DM)[1], which seems to be responsible for developing high viscosity values in rye based diets[2]. The present study provides an approach to understand how a diet based on rye affects feed and digesta viscosity. Additionally the influence of the fermentation technique per se on the viscosity values was investigated.

Methods: To study the impact of rye based diets on digesta viscosity fattening pigs were fed a diet consisting (in % of DM) of 48.8 rye, 29.3 rapeseed meal, 9.80 wheat, 9.80 barley and 2.46 mineral supplement ad libitum for four weeks. The first two groups (per group n = 5) were offered a non-fermented liquid diet. Group three (n = 5) was fed the same diet, after 24 h of fermentation with a starter culture (Schaumalac feed protect XP G). Finally, the last five animals received a liquid diet consisting of 60 % fermented and 40 % non-fermented cereals. The non-fermented cereals were ground by a roller mill only to increase particles' size in the diet. For viscosity measurement, liquid feed and digesta samples (stomach, anterior small intestine, caecum) had to be processed before. Approximately an aliquot of 30 ml was needed. After centrifuging the sample at 10,000 g for 5 min (Heraeus Biofuge Stratos), viscosity was determined by Brookfield Model DV-II + Viscometer, which was equipped with a spindle (S40) rotating at 10 rpm. After centrifugation the measuring unit was filled with 600 µl of the clear particle-free supernatant. Statistical evaluation was done by SAS® (t-Test).

Results: The viscosity measurements revealed a correlation between the feed preparation (non-fermented versus fermented liquid feed) and viscosity values of feed up to digesta over the whole gastro-intestinal-tract. The first two groups were summarized because of the same feeding concept. The viscosity values of the non-fermented liquid feed increased significantly ($p<0.05$) during the gastro-intestinal-passage until the anterior small intestine (feed: 2.40 ± 0.245 mPa*s → stomach: 4.65 ± 1.65 mPa*s → anterior small intestine: 20.3 ± 11.57 mPa*s) and decreased towards the caecum (8.81 ± 4.45 mPa*s). In group three (fermented liquid feed) a completely different course could be observed: The viscosity values of the diet were nearly double compared to the former groups, but during the gastro-intestinal-passage the viscosity values of the digesta decreased significantly ($p<0.05$) in the stomach and increased slowly again towards the caecum (feed: 4.73 ± 0.215 mPa*s → stomach: 1.62 ± 0.217 mPa*s → anterior small intestine: 3.43 ± 0.634 mPa*s →caecum: 5.92 ± 4.19 mPa*s). The last group (only 60 % of the feed was fermented) showed values varying between the other groups.

Conclusion: By feeding pigs a diet based on rye, viscosity values in the diet as well as in the digesta during the gastro-intestinal-passage changes. Further it has to be underlined that also the feeding concept (non-fermented versus fermented liquid feed) had a great impact on viscosity values in the feed as well as in the digesta. Here it should be emphasized that digesta viscosity values are rather low in pigs fed the fermented liquid diet. Lower viscosity values may force enzyme efficacy, which might explain the improved digestibility rates of fermented liquid diet described by BUNTE et al. 2018[3].

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108.

Effects of substitution of wheat by rye in diets for young fattening pigs regarding digestibility and performance

Auswirkungen eines Ersatzes von Weizen durch Roggen im Mischfutter für junge Mastschweine auf Verdaulichkeit und Mastleistung

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Introduction: In the last decades rye was not of high interest for nutritionists / farmers because of possible contaminations by ergot alkaloids and anti-nutritive constituents [1]. Meanwhile there seems to be a renaissance of this cereal. On the one hand the breeding success regarding the pollen plus technology resulted in a lower prevalence of claviceps infection [2] and on the other hand the special constituents that characterize rye (e. g. arabinoxylans and fructans) are reasons for growing interest. This special carbohydrate composition has a positive effect on gut health and also maybe animal welfare [3]. Thus it was of interest to compare groups of young fattening pigs fed with different rye contents in the diet to determine the effect of substituting wheat by rye on several parameters (digestibility / performance) in young fattening pigs.

Methods: 20 pigs (age: 52 ± 2.22 days; bw: 19.5 ± 3.07 kg) were housed individually in four feeding groups of 5 pigs each without litter. Each group received an identical diet consisting of rye, wheat, barley, soy, potato protein and mineral supplement. The sum of wheat and rye was 69 % of the diet, whereby the compound feed of each group was characterized by a different ratio of rye to wheat (1st group: 100 % wheat, 2nd group: 66.6 % wheat and 33.3 % rye, 3rd group: 33.3 % wheat and 66.6 % rye, 4th group: 100 % rye). The pelleted diets were tested on particle size distribution by wet sieve analysis and on the nutrient content by Weender analysis. The animals were fed 4 weeks with the respective compound feed variant ad libitum. Over the entire experimental period, gains (weekly) and feed intake (daily) were determined individually. In addition, during the second week of the study, the feces were collected completely and individually for 5 days to determine the apparent total tract digestibility, at ad libitum feeding.

Results: Unexpectedly there was no systemic effect in the feeding groups with higher rye contents regarding feed intake. During ad libitum feeding digestibility rates did not differ significantly ($p < 0.05$; t-test; SAS® Enterprise Guide®) when the whole share of wheat was substituted by rye (89.3 ± 0.88 against 88.3 ± 0.96). The feed conversion ratio was 1.59 for the 1st group, 1.54 for the 2nd group, 1.58 for the 3rd group and 1.66 for the 4th group. The determined daily weight gain was 884 g for the 1st group, 854 g for the 2nd group, 874 g for the 3rd group and 889 g for the 4th group. Wet sieve analysis shows no significant differences in the proportion of fine particles (particle size < 0.2 mm) in the different diets (38.0 ± 1.54).

Conclusion: Regarding the above mentioned performance parameters, there were no obvious disadvantages replacing wheat by rye. In addition, no negative side effects of rye regarding feces composition in young pigs could be observed. Moreover, there is no restriction with regard to the age of the animals. Consequently, rye can already be used in very young pigs (from 7 weeks onwards) without obvious disadvantages.

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109.

Losses of fecal starch in production dairy herds

Verlust von Stärke im Kot von Milchviehherden

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Introduction: Starch is an important source of energy, particularly in diets offered to intensely managed dairy cattle. The ability of ruminants to utilize starch is, among other factors, impacted by its molecular structure and feed processing method. Mechanical processing of grain reduces particle size and increases the surface area available for microbial attachment and enzymatic hydrolysis (1). Measurements of fecal starch are useful to assess the effectiveness of grain processing and minimize economic losses caused by undigested starch and therefore recommended as a herd monitoring tool for dairy producers (2). However, chemical analysis of starch from fecal samples is expensive and as a result not routinely employed by producers. Consequently, this study was designed to identify useful parameters that can act as predictors for losses of fecal starch in production dairy herds.

Methods: Nineteen dairy farms were included in this study. Participating farms had free-stall barns and fed complete TMR, without offering any other feeds on the side. On each farm, feces from 15 cows was sampled from the barn floor immediately after defecation and pooled by herd. In addition, TMR and feed grains were sampled and the ingredient composition of the TMR was recorded. Feces and TMR were analyzed for DM, organic matter, NDF, ADF and starch. Particle size distribution of the TMR was determined using the Penn State Particle Separator. The particle size distribution of the feces was determined by manual wet sieving using the NASCO Digestion Analyzer (4.8, 2.9, and 1.6 mm screen). To determine the particle size distribution of the grain portion of the diet, processed feed grains were screened dry through 2, 1, and 0.36 mm-sieves. In addition, samples of whole and processed grains were collected to determine the processing index (PI), which is the volume weight of the feed grain after processing expressed as a proportion of its volume weight before processing. To identify factors affecting the fecal starch content, simple and multiple linear regression analyses were performed using the PROC REG procedure of SAS (Version 9.4) with the fecal starch concentration as only dependent variable. Significance was declared at $P<0.05$. The goodness of fit was ranked based on the highest adjusted r^2 (adj.r^2) and lowest root mean square error.

Results: The fecal starch content varied between 2.4 and 28.6 (±7.02 SD) g/kg feces (DM basis) among farms. Dietary inclusion rate of forage or concentrate, particle size distribution, and average particle size of the TMR had no relationship with fecal starch. Increases in the starch content of the TMR (TMRSt; $r^2=0.210$), amount of fecal particles >4.8 mm ($Fpart_{>4.8mm}$; $r^2=0.375$), and grain particles >2 mm ($Gpart_{>2mm}$; $r^2=0.396$) were associated with increases of the fecal starch content ($P<0.05$). In contrast, increases in processing index (PI; $r^2=0.210$) and grain particles >0.36 mm ($Gpart_{>0.36mm}$; $r^2=0.\ 0.338$) were associated with a reduction in fecal starch content ($P<0.05$). The best fit multiple regression model was: fecal starch content (g/kg DM) = -18.23 + 0.06 TMRSt (g/kg DM) + 1.0 $Fpart_{>4.8mm}$ (% DM) + 0.11 $Gpart_{>2mm}$ (% DM) and explained 53.7% (adj.r^2) of the observed variation in fecal starch.

Conclusions: Fecal losses starch in the examined herds were low and no cause of immediate concern. However, TMRSt and the presence of $Gpart_{>2mm}$ were associated with increased losses of fecal starch. Therefore, particularly when feeding rations containing elevated levels of starch, grain processing should be monitored closely. When TMRSt and $Gpart_{>2mm}$ are used in combination with information on the percentage of $Fpart_{>4.8mm}$, fecal losses of starch can be predicted. However, the accuracy of the prediction is not satisfactory, which might be due to the relatively small sample size of this study. More on-farm survey data is needed to validate and improve our prediction model.

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110.

Estimating segmental and total tract apparent digestibility of nutrients in horses using feed-internal and external markers

Schätzung der partiellen und gesamten Verdaulichkeit von Nährstoffen bei Pferden unter Nutzung interner und externer Marker

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Partitioning of apparent digestibility (AD) estimates is helpful to assess the nutritive value of the feed and to identify possible risks for digestive disorders in horses. Investigations on partial AD are less available and less address carbohydrate fractions. Recent studies suggest that huge parts of water-soluble carbohydrates (WSC) are largely decomposed already in the stomach (1, 2), which elevates the risk for mucosa ulceration. The aim was to investigate the AD of DM, proximate nutrients and WSC along the digestive tract using euthanized horses and a variety of internal and external markers.

Methods: 12 Warmblood horses, 534 ± 64.5 kg body weight (BW), 14 ± 7.5 years, were adapted for 20 d to meadow hay (1.5 kg/100 kg BW/d) and crushed oats (1.2 g starch/kg BW/d), offered 2 times/d. Six horses also received 0.15 g/kg BW/d fructooligosaccharides + inulin *via* Jerusalem artichoke meal (JAM), the remainder corncob meal without grains (CMG) as control. Plant-internal 4N hydrochloric acid insoluble ash (AIA), ADL, *n*-heptacosane, *n*-nonacosane, *n*-hentriacontane and *n*-tritriacontane (C33), and Cr_2O_3 (~ 2.8 g) and TiO_2 (~ 2.5 g) offered by bolus 2 times/d (12 d ante mortem), were used as markers. The horses were euthanized at d 21 ~ 1 h after the morning meal. Representative samples of digesta were taken from the stomach (pars nonglandularis and pars glandularis), caecum (CAE), ventral and dorsal colon ascendens, and colon transversum (CT). Faeces were sampled 5 d ante mortem and bulked. DM, proximate nutrients, starch (amyloglucosidase method) and AIA were determined (3). Glucose, fructose, sucrose and fructans were analysed by HPLC, alkanes by GC, Cr_2O_3 and TiO_2 by ICP-OES. AD was calculated for each horse until the end of the individual gut segment and for the total tract. In the hindgut, AD was calculated on daily diet basis, in the stomach considering the morning meal. Statistical analysis was performed using a mixed linear model (SAS 9.4 MIXED), which included fixed segment, marker and treatment effects, a segment × marker interaction, and a random animal effect.

Results: The stomach seemed considerably involved in nutrient degradation with particular high disappearance of simple sugars, starch and fructans (AD up to 78, 74 and 56 %). No WSC were measured in CAE. In the hindgut, AD estimates increased from CAE to CT (DM: 0.49–0.79 with AIA, 0.44–0.56 with C33; CP: 0.51–0.82 with AIA, 0.47–0.63 with C33). Supplying JAM had no effect on AD. AD differences did mainly exist between gut segments ($P < 0.05$) and markers ($P < 0.001$), except for starch AD. All nutrients' AD estimates did less differ among the plant alkanes; they had a vast variation among the animals when based on Cr_2O_3 or TiO_2.

Conclusion: The extensive disappearance of WSC including starch in the stomach should be considered for feed evaluation and risk assessment concerning stomach ulceration, although parts of WSC are possibly not decomposed in the stomach but are rapidly flowing with the liquid phase into the intestine. The probable AD underestimation by ADL confirms the known instability of ADL during the digestive process. AIA seems to overestimate AD along the hindgut, especially for fibre fractions. AD seemed best estimated by cell wall associated plant alkanes. Estimations by Cr_2O_3 and TiO_2 were only accurate for total tract AD.

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This study was part of a project funded by the German Research Foundation (DFG).

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111.

Effect of conjugated linoleic acid and α-linolenic acid on the somatotropic axis of dairy cows fed a diet with reduced n-3 fatty acid content during late pregnancy and early lactation

Einfluss von konjugierter Linolsäure und α-Linolensäure auf die somatotrope Achse bei Milchkühen mit einer reduzierten Versorgung mit n-3 Fettsäuren im Grundfutter während der Spätträchtigkeit und Frühlaktation

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The increasing milk yield in dairy production requires rations with high energy content mainly delivered by corn silage (CS). Diets with CS as single forage source result in a low essential fatty acid (EFA), i. e. α-linolenic acid, and conjugated linoleic acid (CLA) supply (1). First results of the present study indicated an improved energy balance during the transition from late pregnancy to early lactation, when CLA was supplemented in cows fed a CS diet (2). A further objective of the study was to investigate the changes of the somatotropic axis in dairy cows that received a CS diet and were abomasally infused with EFA and CLA during the transition period.

Methods: Forty rumen cannulated German Holstein cows (11000 kg milk/305 d in 2nd lactation) were investigated in 5 blocks of 8 cows, respectively, from wk 9 antepartum (ap) to wk 8 postpartum (pp). The CS based total mixed ration was fed *ad libitum* during lactation (wk 22-6 ap and wk 1-8 pp, 7.1 MJ NEL/kg dry matter (DM)) and dry period (wk 6-1 ap, 6.5 MJ NEL/kg DM). The cows were fitted with abomasal tubes and assigned to one of 4 treatment groups. Cows were daily supplemented from wk 9 ap until wk 8 pp either with coconut oil (CNTR, 76g/d), linseed and safflower oil (EFA, 78 and 4g/d), Lutalin® (CLA, *c9, t11* and *t10, c12* in equal amounts, 10 g/d each) or EFA+CLA. During the dry period, doses of the fat treatments were halved, respectively. Plasma concentrations of insulin, growth hormone (GH), insulin-like growth factor (IGF-I; all by specific radioimmunoassay), and related binding proteins (IGFBP) -2, -3 and -4 (by quantitative ligand blot; 3) 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. Data were analysed using the MIXED procedure of SAS by repeated measurements ANOVA containing treatment, time, block and the treatment × time interaction as fixed effects, as well as calving interval and projected milk yield during 2nd lactation as covariates.

Results: Plasma insulin concentration increased after drying off ($P<0.001$) and decreased after calving ($P<0.001$) in all groups, was highest on d 1 pp in CLA ($P<0.05$) and was higher during the transition period in CLA than CNTR ($P<0.05$). Plasma GH concentration increased during early lactation ($P<0.05$) and tended ($P<0.1$) to be higher in CLA than in EFA and CNTR on d 49 pp. Plasma IGF-I concentration was highest ($P<0.05$) on d 35 ap and decreased ($P<0.001$) in all groups during the transition period until d 14 pp. Plasma IGF-I on d 28, 21 and 10 ap, as well as on d 49 and 56 pp were higher ($P<0.05$) in CLA than in CNTR. Plasma IGFBP-2 showed lower concentrations before than after calving ($P<0.001$) with a peak on d 14 pp and was lower in CLA compared to EFA ($P<0.05$) and CNTR ($P<0.1$) on d 56 pp. Concentration of IGFBP-3 decreased ap ($P<0.001$), reached lowest concentration on d 1 pp and increased afterwards ($P<0.001$). Plasma IGFBP-3 was higher ($P<0.05$) in EFA+CLA on d 21 ap, and in CLA on d 28 pp than in CNTR. The ratio of IGFBP-3 to -2 changed with time ($P<0.001$), reached the lowest level on d 14 pp and increased afterwards ($P<0.001$). The IGFBP-3/-2 ratio was higher ($P<0.05$) in CLA compared to CNTR and EFA during the entire study. Plasma IGFBP-4 concentration decreased ap ($P<0.01$) and tended to be lower ($P<0.15$) in EFA than in EFA+CLA throughout the entire study.

Conclusions: Our data confirmed a greater stimulation of the somatotropic axis in cows with an improved energy balance during the transition period. Cows supplemented with CLA had higher plasma insulin and IGF-I concentrations and a greater IGFBP-3 to IGFBP-2 ratio than CNTR in blood plasma. On the other hand, EFA supplementation had minor effects on the systemic somatotropic axis.

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112.

Influence of the maternal supply with essential fatty acids and conjugated linoleic acid on the energy status of neonatal calves

Einfluss der maternalen Supplementation mit essentiellen Fettsäuren und konjugierter Linolsäure auf den Energiestatus neugeborener Kälber

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Due to the common utilization of corn silage based rations instead of pasture, the availability of α-linolenic acid (ALA) and conjugated linoleic acid (CLA) for dairy cows is often reduced. First results of the present study indicated that an altered maternal supply with fatty acids also affects the fatty acid status of the neonatal calf and influences its glucose and lipid metabolism (1,2). To investigate if these effects were sufficient to modify the calves´ energy status, the energy expenditure and various parameters that influence the neonatal energy metabolism were analysed in this study.

Methods: Thirty-eight calves born from dams fed corn silage based rations were studied during their first 5 d of life. All dams were abomasally supplemented with either coconut oil (CTRL; n=9), linseed and safflower oil (EFA; n=9), Lutalin® (CLA; n=9) or a combination of EFA and CLA (EFA+CLA; n=11) during the last 9 wk of gestation and the following lactation (3). The study was subdivided into 5 blocks with 7-8 calves born per block. During the experimental period, the calves were fed with similar amounts of colostrum from their own dam. Concentrations of glucagon, insulin, insulin-like growth factor (IGF)-I, IGF-binding proteins (IGFBP)-2 and -3, adiponectin and leptin were measured in the calves´ plasma, which was sampled before feeding from d 1 to d 5. On d 3, an intravenous bolus dose of ^{13}C labelled sodium bicarbonate (1 mg/kg BW) was applied after feeding of the morning meal (6% of BW). The enrichment of ^{13}C in blood CO_2 was subsequently measured for 3 h after tracer injection to calculate the postprandial energy expenditure. The data were analysed by repeated measurement ANOVA of SAS. The model included the treatment (EFA, CLA and their interaction), time, treatment × time, block and sex as fixed effects and the duration of supplementation and gestation as covariates.

Results: Plasma concentrations of glucagon, adiponectin and leptin, but not insulin and IGF-I were higher (P<0.05) on d 2 than d 1 after birth. Maternal supplementation with EFA decreased the concentration of plasma glucagon compared to calves, whose dams did not receive EFA, on d 2 (P<0.05). An impact of maternal EFA or CLA supplementation on basal plasma insulin concentration could not be detected. The concentrations of IGFBP-2 and 3 in plasma were not significantly changed by the maternal fatty acid supply. However, the ratio of IGFBP 3 to IGFBP 2 was higher in calves of the EFA group compared to EFA+CLA calves on d 2 (P<0.05). Maternal CLA supplementation affected plasma IGF-I (P<0.05), leading to a lower concentration if dams were supplemented with CLA. Immediately after birth, plasma IGF-I was higher in EFA than in EFA+CLA calves (P<0.01). Calves, whose dams received EFA had higher plasma leptin concentrations compared to groups without maternal EFA supplementation on d 4 and 5 (P<0.05). On d 3, a higher plasma adiponectin concentration was observed if dams received CLA (P<0.05). The postprandial energy expenditure on d 3 was not affected by the maternal fatty acid supply.

Conclusions: The results of the present study indicate changes in the regulation of the neonatal energy metabolism due to maternal EFA and CLA supply before and after parturition. Maternal EFA supplementation seems to stimulate the neonatal energy metabolism, even though there was no treatment effect on the neonatal energy expenditure.

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113.

Studies with the BFH12 cell line as an *in vitro* model for bovine hepatic steatosis

Studien mit der Zelllinie BFH12 als in vitro Modell der Hepatosteatose des Rindes

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Bovine hepatic steatosis is a common metabolic disorder of dairy cows, especially in early lactation. In order to gain a deeper understanding of the mechanisms involved in this condition more research is required. Currently, there are a few models of bovine hepatic steatosis available, including animal models, liver slices or primary hepatocytes. However, due to a lack of appropriate in vitro models studies are limited in terms of predictive power, experimental robustness, phenotypic stability and batch-to-batch reproducibility. In order to address this issue, we established the fetal bovine hepatocyte derived cell line BFH12 (1) sharing important characteristics with bovine hepatocytes such as phenotype and metabolic activity. The aim of this study was to evaluate whether this cell line is an appropriate in vitro model for bovine hepatic steatosis. **Methods:** BFH12 cells were cultured in Williams' Medium E containing 5 % heat-inactivated FBS, 1 % penicillin/streptomycin, 2 mM L-alanyl-L-glutamine, 100 nM dexamethasone and 0.2 U/mL insulin. In order to induce a steatosis-like phenotype the cells were supplemented with one of the following long-chain fatty acids (LCFA): palmitic acid (PA, 60 µM), stearic acid (SA, 50 µM) or oleic acid (OA, 100 µM). After a 24 h incubation period cytotoxicity was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Intracellular fat accumulation was assessed by nile red or oil red O staining and lipid composition was determined by thin-layer chromatography (TLC) and gas chromatography (GC). **Results:** Treatment with fatty acids at the indicated concentrations had no significant effect on cell viability. Lipid droplets of various sizes were observed after LCFA supplementation, indicating an uptake of the fatty acids. OA induced the formation of larger droplets compared to PA and SA. Furthermore, the treatments resulted in higher levels of major lipid classes, including phospholipids (PL), triglycerides (TAG) and non-esterified fatty acids (NEFA). The most pronounced increase was observed for TAG after OA supplementation. Consistent with these results, GC analysis showed that OA is extensively incorporated into TAG in OA treated cells. However, PA and SA are only moderately incorporated compared to control cells. **Conclusion:** BFH12 can aquire a steatotic phenotype by incorporating and accumulating different types of fatty acids. Therefore, the BFH12 cell line may be a useful in vitro model to study bovine hepatic steatosis and its underlying molecular mechanisms.

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114.

Effects of essential fatty acids and conjugated linoleic acid on fatty acid and oxidative status in plasma and hepatic acute phase response in dairy cows fed a diet with reduced n-3 fatty acid content from late pregnancy to early lactation

Einfluss essentieller Fettsäuren und konjugierter Linolsäure auf den oxidativen Status im Blutplasma und die Akute-Phase-Reaktion in der Leber bei Milchkühen mit einer reduzierten n-3 Fettsäureversorgung während der Spätträchtigkeit und Frühlaktation

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Dairy cows are exposed to greater metabolic and oxidative stress around parturition that may impair physiological function and health (1). Essential fatty acids (EFA), namely α-linolenic acid (ALA), and conjugated linoleic acids (CLA) are known for their health-promoting effects and influence biological processes in liver. First results showed only a minor systemic influence of ALA and CLA treatment on the antioxidative status and acute phase proteins in blood plasma (2). The present investigation focuses on the EFA and CLA effects on thiol groups (SH), glutathione peroxidase activity (GPX), reactive oxygen metabolites (ROM) and biological antioxidant potential (BAP) in blood plasma and on the hepatic acute phase response in dairy cows fed a corn-silage based diet with low n-3 fatty acid content from late to early lactation. The hypothesis was tested that, besides systemic effects on the antioxidative status, EFA and CLA treatment may especially affect the hepatic acute phase response in dairy cows around parturition.

Methods: Forty lactating rumen-fistulated Holstein-Friesian cows (11000 kg milk/305 d in 2nd lactation) were investigated in 5 blocks of 8 cows, respectively, and received a corn silage-based total mixed ration during lactation (wk 22-6 before and wk 0-8 after calving, 7.1 MJ NEL/kg dry matter (DM)) and dry period (wk 6-0 before calving, 6.5 MJ NEL/kg DM). The diet was fed *ad libitum*. From wk 9 antepartum (ap) until wk 9 postpartum (pp) cows were daily treated by abomasal infusion either with coconut oil (CNTR, 76g/d), linseed and safflower oil (EFA, 78 and 4g/d), Lutalin® (CLA, *c9, t11* and *t10, c12* in equal amounts, 10g/d) or the combination of EFA+CLA. The dose was halved during dry period. Blood samples were taken on d 63 and 42 ap and on d 1, 28 and 56 pp to determine fatty acid profiles. Plasma concentrations of SH, ROM and BAP and GPX activity in plasma were measured on d -42, 1, 28, and 56 relative to calving. Liver tissue samples were taken on d 63 and 21 ap, on d 1, 28, and 63 d pp to measure haptoglobin (*HP*), serum amyloid A (*SAA*), tumor necrosis factor (*TNF*)-*a* and interleukin (*IL*)-*1* and -*1β* mRNA abundance. The data were analysed by repeated measurements ANOVA of SAS with the fixed effects of treatment (EFA, CLA and their interaction), time, treatment × time and block. Calving interval and milk yield from 2nd lactation served as covariates.

Results: As expected, ALA increased (P<0.001) in blood plasma with EFA supplementation and was higher (P<0.001) in EFA and EFA+CLA on d -42 ap, 28 and 56 pp than in CNTR and CLA. Sum of *c9, t11* and *t10, c12* CLA in plasma was highest in the CLA group (P<0.001) and higher (P<0.001) in EFA+CLA than EFA and CNTR during the treatment period. Plasma concentrations of GPX peaked (P<0.001) in all groups at d 1. Plasma SH concentration was highest on d 1 (P<0.05), decreased towards d 28 pp, increased in EFA+CLA but decreased in CLA on d 56 pp (P<0.1). Plasma BAP concentration was lower pp than ap (P<0.001) and on d 28 lower (P<0.05) in EFA than in CTRL. Plasma ROM concentrations were not affected by treatments. Abundance of *HP* and *SAA* mRNA were both highest (P<0.001) on d 1 pp and both, *HP* and *SAA* mRNA were higher in EFA+CLA than in EFA and CLA (P<0.05) on d 1 pp. Gene expression of *IL-1* and *IL-1β* increased with time (P<0.001 and P<0.05), and *TNF-a* was not affected by time and treatment.

Conclusions: Our data indicated increased oxidative stress around parturition, but parameters measured in this study were barely affected by EFA and CLA treatment. Elevated *HP* mRNA abundance at parturition in EFA+CLA fitted to the elevated HP plasma concentration in these cows (2). The results pointed at an elevated acute phase response around parturition in cows with combined EFA and CLA treatment.

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115.

Effects of essential fatty acids supplementation on the mRNA abundance of the adiponectin system and the antibacterial peptide BSP30a in submandibular salivary glands of dairy cows fed a maize based ration

Effekte einer Supplementation mit essentiellen Fettsäuren bei Milchkühen auf die mRNA Abundanz des Adiponectin Systems und BSP30a in Unterkieferspeicheldrüsen von Milchkühen, die mit einer auf Mais basierenden Ration gefüttert wurden

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Salivary peptides and proteins originate from plasma and salivary epithel cells. It was shown that adipocytes but also salivary glands express adiponectin (AdipoQ). The adipokine has anti-proliferative effects on human salivary gland epithelial cells (1) and increases rat submandibular salivary gland (SMSG) secretion by the activation of both AdipoQ receptors (2). Salivary glands of cattle express BSP30a and other members of the bactericidal/ permeability increasing protein (BPI) family. The antimicrobial peptide BSP30a shows largest concentrations in SMSG, where it is found in relative high quantities, suggesting an important physiological role in bovine saliva (3). A diet low in α-linolenic acid may not cover the need of high yielding dairy cows for this essential fatty acid. In consequence, the cell membrane composition and SMSG function could be affected. We therefore analysed if low essential fatty acids uptake negatively affected the abundance of the AdipoQ system and BSP30a in SMSG.

Methods: Rumen fistulated Holstein cows (n = 40, 11.000 kg milk in 2nd lactation) were fitted with abomasal infusion tubes and studied from 9 wk antepartum up to slaughter at 9 wk postpartum. Cows were fed a TMR based on maize silage and supplemented via the abomasal infusion tubes twice-daily with coconut oil delivering medium-chain fatty acids (76 g/d), linseed-safflower oil mix, delivering n-3 fatty acids (EFA: 78 + 4 g/d, respectively), LUTALIN (content of c9,t11 and t10,c12 conjugated linoleic acids (CLA) in equal amounts; 10 g/d; BASF SE, Ludwigshafen, Germany), or EFA + CLA. SMSG tissue was collected at slaughter and frozen in liquid nitrogen. The mRNA abundance was quantified by qPCR and normalised using qbase. Relative mRNA data were analysed by the mixed model of SAS using treatment as fixed effect and calving interval in addition to milk production during the second lactation as covariates. LSMEANS were compared by Tukey test. Data are presented as LSMEANS ± SEM.

Results: The mRNA of AdipoQ as well as of both receptors was successfully quantified in SMSG of dairy cows. The abundances of the mRNA of the AdipoQ system as well as the BSP30a were not affected by any of the supplements.

Conclusions: All components of the AdipoQ system were shown for the first time in SMSG of dairy cows which indicates a paracrine and autocrine role of AdipoQ in the bovine SMSG. Comparable to other species, the secretory activity of the bovine SMSG might be affected by AdipoQ. The mRNA of the AdipoQ system as well as of BSP30a was not associated with any treatment. Therefore, it is suggested that the regulation of the mRNA abundance of the AdipoQ system and BSP30a in SMSG is not sensitive to a diet low in n-3 essential fatty acids or CLA. On the other hand, these results may point out the importance of these components for SMSG function and saliva composition.

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116.

Effect of conjugated linoleic acid and α-linolenic acid on hepatic glucose metabolism of dairy cows fed a diet with reduced n-3 fatty acid content during late pregnancy and early lactation

Einfluss von konjugierter Linolsäure und α-Linolensäure auf den Glucosestoffwechsel in der Leber bei Milchkühen mit einer reduzierten Versorgung mit n-3 Fettsäuren im Grundfutter während der Spätträchtigkeit und Frühlaktation

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Sufficient glucose availability is vital for transition cow health and high milk production. Endogenous glucose production (eGP) in cows is – among other factors - related to dietary fatty acid supply (1). Diets provided to dairy cows with a high production performance are mainly based on corn silage (CS) and lead to a low essential fatty acid (EFA), e.g. α-linolenic acid, and conjugated linoleic acid (CLA) supply in cows (2). The aim of the present study was to test if abomasal supplementation of EFA and CLA affects hepatic glucose metabolism and eGP in dairy cows fed a diet with CS as single forage source from late pregnancy to early lactation.

Methods: Forty rumen cannulated German Holstein cows (11000 kg milk/305 d in 2nd lactation) were set-up in 5 blocks of 8 cows, respectively, from wk 9 antepartum (ap) to wk 9 postpartum (pp). The CS based total mixed ration was 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). The cows were fitted with abomasal tubes and assigned to one of 4 treatment groups. Cows were daily supplemented from wk 9 ap until slaughtering at wk 9 pp either with coconut oil (CNTR, 76g/d), linseed and safflower oil (EFA, 78 and 4g/d), Lutalin® (CLA, *c9, t11* and *t10, c12*, 10 g/d each) or EFA+CLA. During dry period, each dose was halved. At wk 4 ap and 3 pp, [$^{13}C_6$] glucose was infused (bolus injection of 5.38 µmol/kg of body weight (BW) followed by a constant infusion of 7.53 µmol/kg BW per h for 4 h). Blood samples were taken before and 1, 2, 2.5, 3, 3.5 and 4 h after the start of infusion to measure the ^{13}C enrichment in plasma glucose and blood CO_2 and to calculate eGP and glucose oxidation (GOx). Liver tissue samples were obtained on d 63 and 21 ap, on d 1, 28, and 63 d pp to measure glycogen content and mRNA concentrations of pyruvate carboxylase [*PC*], cytosolic and mitochondrial phosphoenolpyruvate carboxykinase [*PCK1, PCK2*], propionyl-CoA-carboxylase α [*PCCA*] and glucose-6-phosphatase [*G6PC*]. Data were analysed using the MIXED procedure of SAS by repeated measurements ANOVA containing treatment, time, block and the treatment × time interaction as fixed effects, as well as calving interval and projected milk yield during 2nd lactation as covariates.

Results: The eGP was lower and GOx higher ap compared to pp ($P<0.001$), and eGP was lower pp ($P<0.05$) in CLA and EFA+CLA than in EFA. Hepatic glycogen content decreased after calving ($P<0.001$), but tended to be higher ($P<0.1$) in CLA and EFA+CLA than in CNTR on d 28 pp. Abundance of *PC* mRNA increased ($P<0.001$) on d 1 pp, and was higher ($P<0.05$) on d 1 in CNTR than in all other groups. The *PCK1* mRNA abundance was lower ap than pp, and increased with ongoing lactation ($P<0.001$). Abundance of *PCK2* mRNA increased after calving ($P<0.001$), was lower in EFA+CLA than in CNTR ($P<0.05$) on d 1 pp, and highest in CLA on d 28 pp ($P<0.05$). Abundance of *PCCA* and *G6PC* mRNA increased ($P<0.01$) after d 1 pp. On d 28 pp, abundance of *PCCA* was lower ($P<0.05$) in EFA than in CLA, and abundance of *G6PC* mRNA was lower ($P<0.05$) in EFA+CLA than in CLA.

Conclusions: The reduced eGP and elevated liver glycogen content pp indicated a glucose-sparing effect in CLA and EFA+CLA cows to retain glucose homeostasis due to less glucose utilization for milk fat synthesis (1,3). Although mRNA expression of *PCK2*, *PCCA* and *G6PC* were lowest either in EFA or EFA+CLA on d 28, highest eGP pp was measured in EFA cows, pointing at a variable influence of EFA on hepatic glucose metabolism in dairy cows pp. Overall, glucose homeostasis is achieved in CLA cows pp by reducing eGP and in EFA cows by an up-regulation of eGP.

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Do glyphosate-based herbicides have different effects on *in vitro* rumen microbial metabolism and metaproteome compared to pure glyphosate?

Gibt es unterschiedliche Effekte von Glyphosatformulierungen verglichen mit Glyphosat auf den mikrobiellen Stoffwechsel und das Metaproteom im Vormagen in vitro?

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Glyphosate is an organophosphorus compound inhibiting the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) in the shikimate pathway and finally leading to plant death. The fact that EPSPS is as well present in microorganisms and fungi raises the question whether the rumen microbial ecosystem of cattle in animal husbandry can be affected by residues of glyphosate-based herbicides (GBH) in feed.

Materials and Methods: Four RUSITEC runs with twelve fermentation vessels each were conducted according to standard protocol (1) with an equilibration (6 days) and an experimental period (9 days). Nylon bags were filled with 15 g of a mixture of dried grass silage (49.5%), dried maize silage (39.7%), cracked wheat cracked (5.0%), dried soya cake (5.0%) and mineral feed (0.8%). During the experimental period, three dosages (0.1 mg/l, 1 mg/l, 10 mg/l) of glyphosate (monoisopropylamine salt solution; 400 mg/ml**)** and of two GBH (Roundup LB plus and Durano TF; 486 mg/ml) were applied directly into the fermentation vessels daily. The lowest dosage of glyphosate corresponded to the highest concentration measured *in vivo* (2) and the others were chosen as worst case scenarios. The substrate was free of glyphosate and the treatment solutions contained the expected amounts of glyphosate. That means per run, three fermentation vessels served as controls and nine vessels received one of the above mentioned treatment. In total, the runs represented the replicates (n = 4). Redox potentials, pH, SCFA production rates, NH_3-N concentrations were measured and the degradation of crude nutrients were analyzed. The isotope ^{15}N was introduced into the vessels with the buffer solution and served as tracer for microbial protein synthesis to increase ^{15}N proportion, which was analyzed in substrate, feed residues, fermentation liquid, liquid and solid associated microorganisms (LAM/SAM) and the buffer solution by means of elemental analysis and mass spectrometry followed by calculations of assimilated N by LAM and SAM. In addition, protein extracts of LAM and SAM were separately analyzed by metaproteomics. In this approach proteins stemming from the microbial community are identified and relatively quantified by LC-MS/MS and subsequent bioinformatics. Hence, the taxonomic composition and metabolic functions of the community can be analyzed. Effects of treatment, glyphosate concentration, time and their interaction were statistically analyzed using the Mixed Models Analysis (SAS Enterprise Guide). Values were regarded as significant at $p < 0.05$.

Results: The composition of the rumen microbiota did not change; neither did we observe functional effects on protein level. Consequently, none of the metabolic parameters exhibited significant effects of the treatment or the glyphosate concentration. Significant effects of the factor time were found for pH, redox potentials, NH_3-N concentrations, total SCFA production and the molar proportions of propionate, isobutyrate, butyrate, isovalerate and valerate. Altogether, all measured values of these parameters, the degradation of crude nutrients and the microbial protein synthesis were in the physiological range known for RUSITEC experiments.

Conclusion: No negative effects of the GBH or glyphosate alone on rumen microbial metabolism or the microbial protein synthesis were observed *in vitro*. In this point, GBH did not distinguish from the application of glyphosate alone. The metaproteome was not changed in response to the addition of GBH or glyphosate alone.

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118.

Long-term dietary exposure to *Fusarium*-toxin contaminated and sodium sulphite treated feedstuff in pigs and its impact on physiological parameters and vaccination response

Langzeitexposition mit Fusarium-Toxinen kontaminierten und Natriumsulfit-behandeltem Futter beim Schwein und dessen Einfluss auf physiologische Parameter und Impftiterentwicklung

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The effects of *Fusarium* toxins and especially deoxynivalenol (DON) as one of the most relevant toxins under central European production conditions, on porcine physiology and immune system are of particular interest, as pigs are known to respond very sensitive to this toxin. However, controlled *in vivo* trials addressing the adaptive immunity of pigs are scarce. In addition, decontamination strategies may also affect pig health by themselves or in interaction with *Fusarium* toxin contamination. Therefore, the aim of the present study was to address these questions in fattening pigs using an influenza vaccination to depict the responsiveness of the adaptive immune system.

Material and Methods: Ninety-six barrows (BHZP, n=16/diet) with an initial body weight (BW) of 32.5 ± 3.4kg were housed individually in floor pens and fed a barley-wheat based diet containing 10 % of either a control (CON; 0.46mg DON/kg maize) or *Fusarium* toxin-contaminated maize (53.4mg DON/kg maize). Prior to dietary inclusion, maize batches were wet preserved (20% moisture content) with 15g propionic acid/kg maize and one of three sodium sulphite (SoS) levels (0, 2.5, 5.0g/kg maize) for 63-70d. Each of the resulting six feeding groups (CON-, CON2.5, CON5.0, DON-, DON2.5 and DON5.0) were further subdivided: half of the animals were vaccinated against influenza (H3N2, H1N1, H1N2; Respiporc FLU3, IDT Biologika GmbH, Dessau-Roßlau, Germany) or injected with 0.9% NaCl (intramuscular, 1st vaccination experimental week 4, booster week 7). Blood samples were taken directly before the trial start (base level) and in week 4, 7, 8 and 10 from *Vena cava cranialis* and following parameters were analysed: red haemogram and total leukocyte counts (automated haematology analyser), thiamine concentrations (HPLC), sulphate concentrations (flow injection analysis), nitric oxide (NO) using the Griess-assay and vaccine titres (competitive ELISA). For statistics we used proc mixed (SAS 9.4) with mycotoxin (DON vs. CON), SoS treatment (0, 2.5, 5.0g SoS/kg maize), vaccination (influenza vs. NaCl) and their interactions as main factors and time as a repeated measure.

Results: All parameters of the red haemogram were significantly influenced by time, but some showed additional treatment effects. DON significantly reduced red cell distribution width (p_{DON}=0.022) and thrombocytes counts (p_{DON}=0.026). Furthermore, vaccination showed a significant interaction with SoS levels for the mean corpuscular volume (p=0.038) as well as with time for platelet distribution width (p=0.039). However, all these changes were always in the range of their reference values. Total leukocyte counts were influenced by a significant interaction of DON*SoS*time (p<0.01). A significant interaction of DON*SoS was observed for NO production, whereby DON- showed higher NO production compared to CON- and values converged with increasing SoS levels. Thiamine concentrations were significantly influenced by time only (base level: 326, week 4: 175, week 10: 186 µg/L) and sulphate concentrations in plasma remained unchanged by any factor. Pigs receiving influenza vaccination established significant titres one week after the booster, whereas all pigs injected with the placebo were negative at all sampling times. The titre formation was neither affected by DON nor by the detoxification treatment of the maize with SoS.

Conclusion: Although we´ve observed significant changes in some parameters of the red haemogram, these were always minor and well in the physiological range and thus likely of no biological relevance. The determined interaction between DON and SoS level for NO production remains to be elucidated as well as the assessment of its biological relevance due to scarcity of reference values. Our investigation demonstrated that neither dietary DON concentrations of up to 4.3 mg DON/kg diet nor SoS treatment of the feedstuff affected the development of antibody titres after influenza vaccination detrimentally.

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119.

Transfer of perfluoroalkyl acids (PFAAs) and their precursors from contaminated feed into eggs of laying hens

Übergang von Perfluoralkylsäuren (PFAAs) und Vorläufersubstanzen aus kontaminiertem Futter in das Hühnerei

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Per- and polyfluorinated substances (PFAS) comprise more than 3.000 substances, of which a lot are used in numerous industrial and consumer products (Wang et al. 2017a). The most prominent PFAS family are the perfluoroalkyl acids (PFAAs) - highly persistent, bioaccumulative and toxic substances that are directly emitted to the environment or indirectly formed by the degradation of polyfluoroalkyl precursor substances (Wang et al. 2017b). Studies on PFAA precursors indicate their relevance for human exposure. Understanding PFAA precursor transfer from feed to food is necessary for human health risk assessment and an important scientific challenge in PFAS research.

Methods: A feeding study was performed under controlled conditions with 12 laying hens (Lohmann brown). Hens were fed a PFAS contaminated diet for 25 days (exposure period). Afterwards four hens were slaughtered, and eight hens were fed with uncontaminated feed for another 42 days (depuration period) before also being slaughtered. The pelleted feed contained hay and barley that were cultivated on PFAS contaminated farmland in Lower Saxony, Germany. During the feeding study, feed intake was recorded daily; eggs were sampled every second day, separated into egg yolk and egg white and pooled for PFAS analysis. The PFAA concentrations were analyzed by the CVUA-MEL using HPLC-MS/MS. The PFAA analyses were confirmed by the Fraunhofer Institute IME in Schmallenberg using UHPLC-MS/MS and complemented by the Total Oxidizable Precursor method (TOP Assay). The Top Assay rapidly converts PFAA precursors into PFAAs and is a new method for the evaluation of total PFAS (precursors + PFAAs) concentration in feed and food.

Results: Up to eight PFAAs of different chain length (C4-C8) could be detected in feed and egg yolk. The calculations of PFAA transfer into eggs indicated a higher excretion of perfluoroheptane sulfonic acid (PFHpS) and perfluorooctane sulfonic acid (PFOS) than intake via feed. The subsequent TOP Assay of feed showed an increase between 230 to 790 % for PFAAs with chain lengths of five to eight carbons after the oxidation of feed. This is strong evidence for the presence of considerable amounts of precursors. The precursor quantification showed concentrations of 4.8 ± 0.2 µg perfluorooctane sulfonamide (FOSA)/kg and 4.3 ± 0.2 µg perfluorooctanesulfonamidoacetic acid (FOSAA)/kg as well as significant higher amounts of the alkylated substances n-methyl- und n-ethyl-FOSAA (41.3 ± 4.8 µg and 33.0 ± 3.6 µg per kg feed, respectively). Almost all PFAS were detected in egg yolk. PFAA analysis showed the highest PFAS concentration between in the first five days after the end of the exposure period. Thereafter, PFAS concentration decreased. However, perfluorohexane sulfonic acid (PFHxS) and PFOS were not exhaustively excreted until the end of the depuration period. An increase of perfluorooctanoic acid (PFOA), PFHxS and PFOS between 109 to 647 % was determined after the oxidation of the egg yolk samples at day 26 that confirmed the authors' hypothesis of the precursor being transferred from feed to eggs. The precursor target analysis of egg yolk identified FOSA with concentrations of 8.0 ± 0.1 µg/kg egg. The highest measured concentration was detected for FOSAA with 60.6 ± 5.5 µg/kg followed by n-methyl-FOSAA at 50.8 ± 0.8 µg/kg egg. The TOP Assay of the egg yolk shows an increase in the concentration of PFHxS, PFOA and PFOS and all precursors. The most pronounced increase in concentration was exhibited by FOSAA.

Conclusion: The results of the precursor analysis suggest the following first hypotheses: i) The transfer of non-alkylated precursors into the egg of laying hens is more extensive and / or ii) alkylated precursors (n-methyl- / n-ethyl-FOSAA) are dealkylated in the laying hen / egg. Regardless of derived hypotheses, the results clearly show that the precursors are important contributors for PFAS contamination in feed, being able to transfer into eggs.

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120.

Effects of glyphosate residues and different concentrate feed proportions in dairy cow rations on the ruminal microbial community

Einflüsse von Glyphosatrückständen und variierenden Kraftfutteranteilen in Milchkuhrationen auf das ruminale Mikrobiom

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Introduction: Glyphosate is the most used active substance in non-selective herbicides in agriculture worldwide (1). Different studies discuss a concentrate dependent influence of glyphosate on the ruminal microbial community based on *in-vitro* experiments. However, information about the influence of glyphosate on the ruminal microbiota *in-vivo* is still scarce. This study focused on the influence of a real life worst-case scenario of glyphosate contamination in feedstuffs on the ruminal microbiome in the context of different concentrate feed proportions in a total mixed ration (TMR) in dairy cows.

Material & Methods: After an adaption period with 45% concentrate feed proportion in the TMR, 64 German Holstein Cows in late lactation were split into four groups. Each group received a specific diet with a combination of a glyphosate containing TMR (GL (uptake > 73 mg/d)) or an uncontaminated TMR (CON (uptake < 1 mg/d)) and either 60% (HC) or 30% (LC) concentrate proportion in the TMR (Feedstuff preparation, glyphosate uptake and carry over are available in (2)). For microbiome analyses, ruminal fluid was collected at the beginning of the trial, after 8 weeks and after 16 weeks. Total DNA was extracted from the ruminal fluid and V4/V5 regions of the 16S rDNA were amplified using primers Com1/Com2-ph (3). Sequencing data was analyzed using Qiime2, while further statistical analyses were conducted using SAS.

Results: At the beginning of the trial, all groups showed a similar alpha diversity of the ruminal microbiome. After 8 weeks and 16 weeks, for the HC groups a significant decrease in microbial alpha diversity and for the LC groups a significant increase in microbial alpha diversity were observed. A comparison of GLY and CON groups showed no significant differences between the groups concerning alpha diversity. For beta diversity metrics it was observable, that three clusters were formed based on the concentrate feed proportion in a PCoA analysis. One cluster consisted of the samples from the adaptation phase, the LC groups formed a second cluster and the HC groups formed the third cluster. Glyphosate however did not influence the clustering. Moreover, in an analysis of the taxonomic profiles, significant changes in the abundance of key microbial taxa could be linked to changes in the concentrate feed proportion. This goes in line with an observed difference in the ratio of the most abundant bacterial phyla Bacteroidetes and Firmicutes between the HC and LC groups. Finally, changes in the composition of ruminal SCFA could be correlated with changes of taxa from the Bacteroidetes and Firmicutes. However, no significant differences in detected microbial taxa were observed for the GLY and CON groups.

Conclusions: The concentrate feed proportions in TMRs influenced the composition of the ruminal microbiome. High proportions of concentrate lead to a less diverse microbial community dominated by key taxa. This is possibly caused by less complex fermentation patterns from concentrate feed, which can be completed by a small set of bacterial taxa. In contrast high proportions of roughage lead to a more even and complex microbial community. This could be related to more complex fermentation processes, which in turn involve a complex microbial community performing the different fermentation steps. These findings are underlined by observed changes in the taxonomic composition, which clearly correlate with SCFA proportions in the ruminal fluid that are typically linked to concentrate feed proportions. However, glyphosate residues in feed did not influence the ruminal microbiome in its core functions *in-vivo*. This supports *in-vitro* findings, which show glyphosate effects only when using glyphosate concentrations that are significantly higher than expected for a real life scenario. This could be based on high amino acid availability in the rumen, which helps bypassing a temporary glyphosate-induced auxotrophy for aromatic amino acids in microorganisms.

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121.

Excretion of non-dioxin like polychlorinated biphenyls (ndl-PCBs) in blood and milk of accidentally exposed dairy cattle and transfer to the calf

Ausscheidung von nicht-dioxinähnlichen polychlorierten Biphenylen (ndl-PCB) in Blut und Milch von Milchkühen nach unbeabsichtigter Exposition und Übergang in das Kalb

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Polychlorinated biphenyls (PCBs) are environmentally persistent substances accumulating in the adipose tissue of exposed animals and humans. Food of animal origin represents the main route of human exposure to PCBs. Non dioxin-like (ndl) PCBs have a toxicological profile different from that of dioxin-like (dl) PCBs, with liver and thyroid toxicity observed for single congeners in the animal model. Data on the excretion of ndl-PCBs is scarce. Monitoring authorities in Lower Saxony, Germany, had detected elevated levels of ndl-PCBs in dairy milk. The contaminated milk had been traced back to one dairy farm, where a confirmatory analysis had yielded a level of 132 ng ndl-PCB/g milk fat, exceeding the maximum authorised level for ndl-PCBs in milk of 40 ng/g fat (Reg. [EU] No. 1259/2011) by a factor of three.

Methods: Three cows (crossbreds beef x dairy) with highest ndl-PCB excretion into milk were identified and transferred to the research station of the German Federal Institute for Risk Assessment (BfR) in order to monitor ndl-PCB levels in blood and milk under controlled conditions for up to 288 days. Cows were fed an ndl-PCB-free total mixed ration. Blood was sampled for ndl-PCB analysis at four time points ante partum (a.p.) and on days 0, 14, 28, 56 and 84 post partum (p.p.). Milk samples were analysed for ndl-PCBs in two-week intervals a.p. and on days 0, 7, 14, 21, 28, 42, 56, 70 and 84 p.p. Two calves were raised with their mother´s milk over twelve weeks and blood ndl-PCB levels were determined on days 0 (prior to colostrum uptake), 14, 28, 56 and 84. Six indicator congeners (PCB 28, 52, 101, 138, 153 and 180) were analysed by GC/HRMS.

Results: Initial blood levels (sum of six indicator congeners) were 611, 119 and 140 ng ndl-PCB/g fat in cows 1, 2 and 3, decreasing by 82%, 79% and 77%, respectively, within the following 100 days. During the dry period, levels increased marginally. After parturition, blood levels continued to decrease by 70% (cow 1) and 39% (cow 2) to reach levels of 46 and 24 ng/g fat, respectively, when the experiment was terminated on day 84 p.p. At parturition, the blood ndl-PCB level of cow 1 was 152 ng/g fat, with a level of 123 ng/g fat in her calf resulting from placental transfer. Respective figures in cow and calf 2 were 32 ng/g fat and 58 ng/g fat. In the case of calf 1, intake of milk made blood levels increase for the following four weeks, reaching 197 ng/g fat on day 28. From then on, levels decreased to 103 ng/g fat on day 84. In calf 2, blood levels decreased over time, with levels in the calf constantly exceeding levels in the cow by a factor of roughly two. In accordance with blood levels, milk was highly contaminated in cow 1 (643 ng ndl-PCB/g milk fat at initial sampling). Until drying off after 97 days levels decreased to 29% of the initial level with the steepest decline during the first six weeks. At parturition, milk contained 177 ng ndl-PCB/g fat with a further slow decrease to 10% of the initial level (66 ng/g fat) when the experiment was terminated. In cow 2, milk levels were 122 ng/g fat initially and 37 ng/g fat at parturition. Development of excretion over time was similar to cow 1, with levels decreasing to equally 29% until day 97 (drying off) and a final level of 17 ng/g fat (14% of initial level). Cow 3 was milked continuously for 184 days. The initial level was 181 ng/g fat and levels decreased to 19 ng/g fat until day 97 with almost constant levels from then on.

Conclusion: In case of high contamination, milk ndl-PCB levels exceeded the maximum level even after a study period of 288 days. The gained data can be used to plan controlled studies on the toxicokinetics of ndl-PCBs in dairy cows and to develop computer-based tools for risk management.

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122.

Study on the transfer of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) into milk of high-yielding cows for the determination of dioxin half-lives in milk in consideration of cows' metabolic state

Transferstudie mit Milchkühen zur Ermittlung von Dioxin-Halbwertszeiten in der Milch unter Berücksichtigung der Stoffwechsellage

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An experimental transfer study of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans **(**PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) into the milk of high-yielding cows has been performed in a collaboration project between the Max Rubner Institute (MRI), the Federal Research Institute for Animal Health (FLI), the German Federal Institute for Risk Assessment (BfR) and the Christian-Albrechts-Universität Kiel (CAU). The objectives are the determination of toxicokinetic parameters in consideration of the metabolic state of cows and the derivation of congener-specific transfer rates and half-lives ($t_{1/2}$)[1] adapted to current conditions in practice. The first results will be presented for the transfer of PCDD/Fs into milk at catabolic and anabolic metabolism. Although many studies exist in the literature on the transfer of PCDD/Fs into milk, we have found them to be of limited applicability for risk assessment. Typical issues with published studies include missing steady-states, different dosages, types of administration, duration of exposure or differing dairy cattle breeds, etc. Consequently, no coherent transfer parameters can be derived from them. This situation prompted the realization of the current project.

[1] time required for PCDD/F concentration in milk (pg/g fat) to reduce to half its initial value

Methods: A feeding study was performed with high-yielding cows (Friesian Holstein) in their second lactation. Next to four control animals fed uncontaminated feed, five experimental animals (experimental group) were daily exposed to a PCDD/F-PCB-mixture with an average of 6.3 ng WHO-PCDD/F-TEQ/kg feed (DM) administered in a gelatin capsule during the morning feeding time during two non-consecutive periods. The exposure dose was calculated assuming a dry matter intake of 24 kg/cow/day. The first exposure period started with the first day after parturition (days 1-28 p.p.). This was followed by a depuration period which end depended on the duration of each cow reaching the anabolic state (period 1). The second exposure period began when the animals were in a state of anabolic metabolism. This was also followed by a depuration period until the end of the lactation period (period 2). The trial ended with the euthanasia of all animals. The metabolic states were confirmed by measuring the fat deposit of each animal according to the method of Raschka (2015) using ultrasound and were complemented by analyses of fatty acids in milk. Throughout the feeding study, all animals were fed a diet containing grass silage, maize silage and straw as well as concentrates individually according to milk yield. For each cow, an aliquot of milk was taken and pooled per day for analysis of PCDD/Fs concentration and milk composition. T-test was used for statistical evaluation.

Results: In period 1 (catabolic metabolism) the congener specific transfer rates tended to be higher than in period 2 (anabolic metabolism). However, the elimination kinetic with an average $t_{1/2}$ of 4 days during α-phase (fast elimination) and 39 days during *β*-phase (slow elimination) did not differ among the two metabolic states. One exception was 123478-HpCDF, which had a significantly shorter $t_{1/2}$ during the anabolic state (*p*-value: 0.019). The evaluation of $t_{1/2}$ during the *α*-phase indicates a significant shorter $t_{1/2}$ for 1278-TCDF and 12378-PeCDF (each 1.5 days) compared to the average, and the longest $t_{1/2}$ for 1234678-HpCDD (7.4 days). During *β*-phase, 1278-TCDF and 12378-PeCDF also showed shortest $t_{1/2}$ in milk (15-18 days), whereas the longest $t_{1/2}$ of 63 days was calculated for 1234678-HxCDF.

Conclusion: For the first time, a complete profile of the elimination half-lives in the *α*-phase and *β*-phase were obtained for PCDD/Fs in milk. However, further experimental parameters will be included for extended statistical evaluation in order to verify the accuracy of the above Results.

1) RASCHKA C. (2015): Quantifizierung unterschiedlicher Fettdepots mittels Ultrasonographie und deren Entwicklung in der Transitperiode unter dem Einfluss von Niacin bei Milchkühen. Dissertation Tierärztliche Hochschule Hannover

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Workshop
Vitamins in Ruminants

Vitamin D – Too soon to turn off the light

Vitamin D – Zu früh, um das Licht auszuschalten

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Vitamin D functions as a hormone in humans and a variety of animal species. It can be obtained from food or by endogenous synthesis. Its de novo synthesis requires the exposure of 7-dehydrocholesterol in the skin to ultraviolet light in the wavelength range from 280 to 320 nm (UVB). Humans, herbivorous mammals, birds, reptiles and fish can use both strategies to acquire sufficient vitamin D (1-6).

Regulation of vitamin D. Vitamin D obtained from the diet or by skin synthesis is then bound to vitamin D-binding proteins. Vitamin D is activated by two enzymatic hydroxylation steps in the liver and kidney: it is first converted in the liver to 25-hydroxyvitamin D (25(OH)D) and then further hydroxylated by the kidneys to form calcitriol (also known as 1,25-dihydroxycholecalciferol), the biologically active form of vitamin D. The synthesis of calcitriol is catalysed by renal 1α-hydroxylase (CYP27B1) and is responsible for the majority of circulating calcitriol. The expression of CYP27B1 is strongly upregulated by parathyroid hormone (PTH), which is a signal of calcium status, and downregulated by fibroblast growth factor 23 (FGF23), which is a signal of serum phosphorus. Other factors that are involved in calcitriol regulation are insulin-like growth factor type I (IGF-I), which upregulates the renal synthesis of calcitriol, whereas calcitriol itself can downregulate CYP27B1 in a negative regulatory feedback loop. Calcitriol is also capable of stimulating calcitriol-24-hydroxylase (CYP24A1), an enzyme that degrades calcitriol and 25(OH)D to the inactive form $24,25(OH)_{2D3,}$ which is destined for excretion (7).

In addition to the kidney, keratinocytes and most immune cells are also capable of producing calcitriol via CYP27B1 but not of releasing calcitriol into the circulation. They produce active vitamin D for their own needs. The regulation of calcitriol production in these cells is completely different from that in the kidney. In keratinocytes, macrophages and other immune cells, calcitriol is stimulated by interferon-γ and tumour necrosis factor-α (8-10).

Comparatively little information is available concerning vitamin D absorption and factors that modulate the intestinal uptake of vitamin D. Reboul and co-workers were the first to find that the intestinal absorption of vitamin D involves cholesterol transporters such as Niemann-Pick-C1-like-1 (11). In an experimental mouse study in which we used deuterium-labelled vitamin D, we were able to show that orally administered ezetimibe, a pharmaceutical inhibitor of Niemann-Pick-C1-like-1, reduced the vitamin D concentration in the liver by 80% and the concentrations in the kidney, heart and adipose by 50% while maintaining a normal serum level of 25(OH)D, within 6 weeks (manuscript in preparation). These data suggest that Niemann-Pick-C1-like-1 is an important molecule for the intestinal absorption of vitamin D. The study further showed that circulating 25(OH)D remains stable over a long period of time despite markedly reduced vitamin D concentrations in the body. It is well known that dietary fat is necessary for vitamin D absorption. Recent data from humans showed that vitamin D supplemented in combination with monounsaturated fatty acids leads to higher circulating levels of 25(OH)D than vitamin D supplemented in combination with polyunsaturated fatty acids (12). We were able to show that oral intake of 7-dehydrocholesterol can markedly increase the vitamin D content in different tissues (13).

Biomarker of vitamin D status. The assessment of vitamin D status is currently based on measuring circulating 25-hydroxyvitamin D (25(OH)D). It is assumed that 25(OH)D is a good surrogate measure of vitamin D status to predict health outcomes. It is further assumed that the conversion rate of vitamin D to 25(OH)D in the liver is not tightly regulated and that increases in vitamin D in the body result in increases in the plasma concentrations of 25(OH)D. However, our own data raise considerable doubts regarding the assertion of unregulated hydroxylation. Data from mouse studies show that the plasma concentration of 25(OH)D does not necessarily correlate with the vitamin D content in liver and other tissues (13,14). However, calcitriol is even less suitable as a biomarker of vitamin D because it is very tightly regulated and has an extremely short half-life.

Function and assessment of vitamin D requirement. To assess the daily requirement of a nutrient, it is necessary to have suitable outcome measures or functional parameters that specifically indicate deficiency and that are sensitive enough to discriminate among adequate, insufficient (reduced storage and increased risk of clinical signs) and deficient status. To elucidate the vitamin D requirements, it is important to know the

function of this vitamin in the body. Calcitriol exerts its function through a vitamin D receptor, a transcription factor that modulates the expression of a series of vitamin D-dependent genes in vitamin D-target cells. Gene array analyses reveal that 300-800 genes are regulated by calcitriol, although some of these genes are also regulated by other modulators that can compensate for some effects of calcitriol (reviewed by 15).
Calcitriol regulates the transepithelial transport of calcium in the intestine and kidney. The gene products that are involved in this transport and that are regulated by calcitriol include the apical calcium ion channel proteins TRPV5 and TRPV6, soluble intracellular components such as the calbindins (D9K and D28K), and the intestinal basolateral ATPase-driven calcium pump PMCA1b and/or the renal sodium calcium exchanger NCX1 (16,17). It is further assumed that 30% of phosphate absorption in the intestine depends on vitamin D (18).
In bone, the impact of calcitriol is complex. Calcitriol appears to play an anabolic and catabolic role in bone remodelling because calcitriol stimulates the expression of gene products in osteoblasts that are necessary for the production and mineralization of the osteoid matrix and simultaneously stimulates the expression of proteins that promote the differentiation, activation, and survival of bone-resorbing osteoclasts (19). Osteomalacia is the classical vitamin D deficiency disease. It is characterized by a mobilization of calcium from the mineralized bone surfaces and by an increase in the mass of unmineralized osteoids. Busse and co-workers found that increases in the osteoid surface area are associated with a decrease in bone surface area that can participate in remodelling (20). Consequently, the calcium within the osteoid frame cannot be resorbed and accumulates. Thus, osteomalacia is characterized by a juxtaposition of unmineralized and hypermineralized bone mass, which in turn increases the risk of bone fracture.
Another function of vitamin D is the modulation of innate and adaptive immune responses. The vitamin D receptor is expressed in numerous immune cells (B cells, T cells and antigen-presenting cells). These cells are all capable of synthesizing calcitriol, which appears to be essential for normal immune function (reviewed by 21,22). Insufficient vitamin D levels may lead to dysregulation of the immune response. Data from two systematic meta-analyses of randomized controlled human trials, including results from 10,933 and 5,660 individuals, show an inverse association between vitamin D status and respiratory tract infections (23,24).

An important question is to identify the central function of vitamin D that could be used to determine the need. Difficulties in the assessment of the vitamin D requirement can be summarized as follows:

(i) In humans, more than 80% of all vitamin D in the body comes from endogenous synthesis. Thus, it is difficult to elucidate the amount of dietary vitamin D necessary to meet the requirement.

(ii) The circulating level of 25(OH)D is currently used as a parameter to differentiate among adequate, insufficient or deficient vitamin D status. The Institute of Medicine classified a 25(OH)D plasma level of 50 nmol/l as sufficient to prevent bone disorders (25). Studies that consider further vitamin D-associated diseases, such as immune diseases, recommend 25(OH)D levels of at least 75 nmol/l (26,27).

(iii) The bone disease in adults caused by vitamin D deficiency is osteomalacia. However, there is no specific blood parameter to indicate the presence of osteomalacia, and dual-energy X-ray absorptiometry (DEXA) cannot distinguish between age-related osteoporosis and osteomalacia.

(iv) Disturbances in calcium metabolism as a consequence of inadequate vitamin D cannot simply be analysed by serum calcium because it is homeostatically regulated. Another option is the analysis of PTH, which mobilizes calcium from bone. However, data on PTH levels are difficult to interpret. Some data found that patients with vitamin D deficiency have PTH levels that are still in the normal reference range (e.g., 28). A recent review stated that a serum 25(OH)D level of 75 nmol/l should be used as the defining level for vitamin D insufficiency because in the studies analysed in that review, serum PTH showed a plateau at a serum 25(OH)D of approximately 75 nmol/l (29).

(v) Another difficulty concerns the measurement of 25(OH)D. Currently, different analytical systems are used, such as enzyme-linked assays, radioimmunoassays, and HPLC-based and LC-tandem mass spectrometry-based Methods: However, the results from these methods are not directly comparable.

Conclusion: Vitamin D has multiple functions in the body. The dietary needs and the optimal serum concentrations of 25(OH)D to address all these vitamin D-dependent functions remain a matter of debate. It is likely that the estimated requirement for vitamin D depends on the physiological measures selected.

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Vitamin D Supplementation & Vitamin D Metabolism– Unexpected Findings

Vitamin D Supplementierung und Vitamin D Metabolismus – Unerwartete Ergebnisse

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Use of vitamin D metabolites in livestock

Animals are treated parenterally with vitamin D (cholecalciferol) in order to compensate for deficiencies, during periods of enhanced demand or to prevent periparturient hypocalcemia of dairy cows. The recommended dosage for this purpose varies between 250 and 500 µg/kg body weight (1, 2).

In addition, cholecalciferol is used as a feed additive in livestock to support Ca homeostasis in high performing dairy cows, laying hens, lactating sows and fast growing pigs and poultry. According to the Regulation No 1831/2003 of the European Parliament cholecalciferol can be added to the ration to an amount up to 250 µg/kg (milk replacer for calves and piglets), 100 µg/kg (cattle, sheep and horses), 125 µ/kg (chicken for fattening and turkeys), 80 µg/kg (other poultry) or 50 µ/kg (pigs). To date, 25-hydroxycholecalciferol (25-OHD) is only approved for poultry and pigs. The above mentioned maximum permitted levels are applied for the sum of cholecalciferol and 25-OHD if the hydroxylated metabolite is used.

In recent years, several studies focusing on beneficial effects of 25-OHD in ruminants have been carried out. It has been shown that 25-OHD can stabilize Ca homeostasis around parturition in dairy cows (3), increase the apparent digestibility of Ca in sheep (4), enhance the retention of Ca and P in lactating cows (5) and beef cattle (6) and improve meat quality (7).

Interferences with age

In a study investigating the supplementation of dairy cows with either 4 mg or 6 mg 25-OHD, it could be demonstrated that plasma half-life of 25-OHD was significantly longer in cows entering the 2nd lactation in comparison to older animals. Moreover, the increase in plasma concentrations of 25-OHD during dietary supplementation differed with age. As no correlations between the plasma half-life of 25-OHD and the plasma concentrations of the biologically active metabolite, 1,25-dihydroxycholecalciferol (1,25-(OH)2D or parathyroid hormone (PTH) were found, it was concluded that age has a direct effect on the metabolism of vitamin D (8). This hypothesis is supported by the observation that the activity of the inactivating enzyme 24-hydroxylase (CYP24A1) is lower in younger rats than in older ones (9).

Interferences with other dietary factors

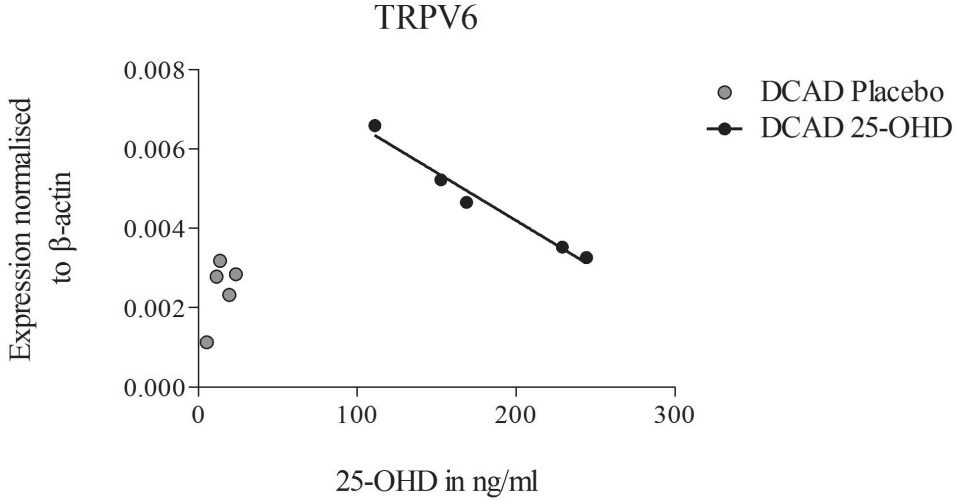
Although the underlying mechanisms are not completely understood, it is well known that high dietary contents of P in late gestation have a negative impact on pariparturient Ca homeostasis in dairy cows (10, 11). Feeding a low P diet in late gestation resulted in significantly increased plasma concentrations of a bone resorption marker and decreased PTH concentrations. At parturition, Ca homeostasis was improved and the synthesis of 1,25-(OH)2D was stimulated despite the lower PTH peak in P-depleted cows (12). In this context, fibroblast growth factor 23 (FGF23) a bone-derived phosphatonin that is positively regulated by plasma phosphate might be of relevance. FGF23 inhibits the enzyme crucial for the transformation of 25-OHD to 1,25-(OH)2D, the 1α-hydroxylase (CYP27B1) (13). In P-depleted cows, FGF23 plasma concentrations should be diminished. In combination with the sudden decrease in plasma Ca at parturition, the low FGF23 plasma concentrations should result in an additional stimulation of 1,25-(OH)2D synthesis independently of PTH.

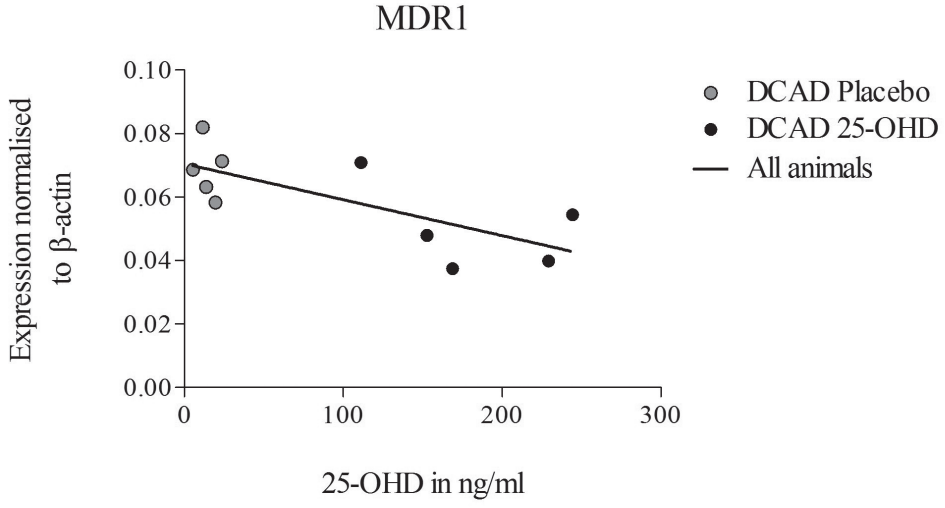
Furthermore, vitamin D metabolism can be influenced by dietary protein content. In young goats fed a low nitrogen ration, plasma concentrations of 25-OHD were increased while those of 1,25-(OH)2D were decreased in comparison to control animals kept on adequate nitrogen supply, even when the Ca content of the diet was restricted, too (14). It was concluded that these effects occurred due to a down-regulation of CYP27B1 mediated by decreased plasma concentrations of insulin like growth factor 1 (15).

A common approach to prevent periparturient hypocalcemia in dairy cows is to decrease either the Ca content or the Ca availability by adding Ca binders such a zeolite A to the prepartum ration. However, this treatment has an impact on P and Mg plasma concentrations, too (16). And although results obtained in studies carried out using cows were encouraging, an effect on the synthesis of 1,25-(OH)2D and the expression of gastrointestinal Ca transport proteins as shown in goats cannot be excluded (17).

Interferences with high plasma concentrations of vitamin D metabolites due to supplementation

The combination of a supplementation with 25-OHD with a ration low in dietary cation anion difference (DCAD) was shown to have beneficial effects on the periparturient Ca homeostasis in dairy cows. But interestingly, animals supplemented the same way but fed a ration high in DCAD had the lowest plasma Ca concentration at parturition (3). A possible explanation for this observation might be a delay of the synthesis of 1,25-$(OH)_{2D}$ and hence impaired absorption of Ca and/or bone mobilisation. Indeed, it was shown in sheep on the same treatment protocol that the high plasma concentrations of 25-OHD due to the supplementation decrease the expression of CYP27B1 while the inactivating enzyme CYP24A1 was up-regulated (unpublished results). Combining the supplementation with a low DCAD diet might overcome these negative effects on vitamin D metabolism by increasing the tissue responsiveness to PTH (18).





But even when combined with a low DCAD ration, supraphysiological plasma concentrations of vitamin D metabolites might have some undesired side-effects. Although the intestinal expression of the Ca transporting protein TRPV6 (transient receptor potential vanilloid type 6) was stimulated in supplemented sheep, a negative correlation between its expression and the plasma levels of 25-OHD could be revealed. Further research is needed to clarify whether this could be a direct effect of 25-OHD or a mechanism mediated by other vitamin D metabolites. Such a potential counterregulation could explain why other studies on the supplementation with 25-OHD could not reproduce the beneficial effects observed in dairy cows by our group (19). Excessive 25-OHD is inactivated to 24,25-(OH)2D, a metabolite that has been shown to be associated with periparturient paresis in dairy cows (20, 21).
In addition, intestinal expression of MDR1 coding for the efflux transporter P-glycoprotein (P-gp) was negatively correlated with plasma 25-OHD. As hepatic protein expression of P-gp was also significantly down-regulated in 25-OHD supplemented sheep, interactions between vitamin D metabolites and the absorption or secretion of xenobiotica cannot be excluded (22).

Conclusions

The optimal level of vitamin D is determined by several factors that are still not fully understood. Especially if high dosages are supplemented, veterinarians and nutritionists have to keep in mind that vitamin D is a precursor of a steroid hormone and thus very likely to induce feedback mechanisms.

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The Role of Vitamins for Optimizing Performance and Health of Ruminants

Die Rolle der Vitamine für die Optimierung von Leistung und Gesundheit der Wiederkäuer

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Introduction

Vitamins have been classically defined as a group of organic compounds, required in minute amounts for the normal development and functioning of body processes such as growth, development, maintenance and reproduction. Although, knowledge about vitamins has evolved considerably since their discovery early in the last century, the quantitative determination of the vitamin needs of cattle has been difficult. Current vitamin requirements of ruminants are based on research data that are at least twenty to thirty years old. Recommendations, derived from these results, are generally close to the minimum levels, required to prevent overt clinical deficiency signs. Yet, the cattle industry must be interested in recommendations for dietary vitamin supplementation, which permit optimum animal health, performance and product quality under commercial conditions. The aim of this review is to summarize research related to the importance of the vitamins A, E and D as well as biotin for ruminants in order to better adapt their vitamin nutrition to the actual needs.

Vitamin A (Retinol) and beta-Carotene

Vitamin A is essential for normal vision as it is integrated into the visual pigment rhodopsin, one of the photoreceptors. But vitamin A supports also the development and the integrity of epithelia, mucous membranes as well as of the skeleton, since it operates as a regulator of cellular growth and differentiation. In farm animals vitamin A is important for normal feed intake, growth and performance. Furthermore, it supports an optimum immune response and thereby improves the resistance against infectious diseases. Vitamin A occurs in three forms: retinol, retinal and retinoic acid. These retinoids are potent signaling molecules, playing among others a central role in the modulation of the immune response at early lactation of ruminants (1).

Vitamin A deficiency in cattle induces loss of appetite, growth retardation or inhibition, loss of vision, defects in bone growth and maturation, reproductive failure and eventually can end in death. Defects in growth and differentiation of epithelial tissues frequently lead to keratinization, resulting in loss of protective functions in the genital, reproductive, respiratory and urinary systems, which increase the susceptibility to infection. Keratinization of epithelia in the digestive tract depresses gland activities and results in poor absorption of nutrients.

Retinol as such does not occur in plants, but its precursors, the carotenes, are present in several forms in green leaves and to a lesser extent in corn grain. Therefore phylogenetically ruminants used to cover their vitamin A requirement via the provitamin A beta-carotene. The primary site of carotene conversion to retinol is the intestinal mucosa, but ruminants do absorb substantial amounts of beta-carotene as well. Normal processes of fat digestion and absorption and adequate dietary fat content are required for the absorption and subsequent conversion of beta-carotene to retinol. Beta-carotene is cleaved by the enzyme 15,15′-dioxygenase, yielding one molecule of retinaldehyde, which is enzymatically reduced to retinol. Beta-carotene is contained in pasture grass, but its content in fresh and dry forage is variable and depends on the conditions during growing, harvest and storage, which makes this source unreliable. Fatal vitamin A deficiency in grazing cattle has recently been observed in Australia after a serious drought, were the grass was found to be low in beta-carotene (2). Since ruminants can absorb and metabolize retinol, their physiological requirement is usually covered by dietary vitamin A. Yet, it has to be taken into account that appreciable amounts of vitamin A (40% to 70%; 3) are degraded in the rumen. The primary site of vitamin A and carotene absorption is the proximal jejunum. Dietary retinyl esters are hydrolyzed to retinol by pancreatic esterases in the small intestine and then absorbed as the free alcohol in association with lipid micelles. After re-esterification to retinyl palmitate in the mucosa, the retinyl esters are transported via the lymphatic system to the liver, where they are stored in parenchymal cells. Hydrolysis of the stored retinyl esters liberates retinol that is combined with retinol-binding protein for secretion into the bloodstream.

Disease resistance is a key feature of vitamin A, which is required for the development and function of immune cells and the maintenance of the protective capacity of mucous membranes and epithelial surfaces. The

effects of both clinical and subclinical deficiencies of vitamin A on the production of antibodies and on the resistance of different tissues to microbial or parasitic infections have been clearly demonstrated (4). Vitamin A has also been used as an adjunct in treating ringworm (Trichophyton verrucosum) infestations in cattle. Both vitamin A and beta-carotene were found to be important for the prevention of certain diseases such as bovine mastitis (5). Furthermore, improved mammary health in supplemented dairy cows during the dry period (6) and lactation (7) and lower milk somatic cell counts during lactation (8) was reported.

Milk production is dependent on vitamin A. Studies in neonatal calves show important interactions between vitamin A, lactoferrin and insulin-like growth factor (IGF) binding proteins (9; 10). Furthermore vitamin A is important for mammary gland epithelial cell proliferation and apoptosis during the dry period, which beneficially influences lactation performance due to the fact that milk production is dependent on the number of mammary secretory cells and on their synthetic and secretory capacities. Also supplemental beta-carotene was observed to increase milk production (11), indicating that beta-carotene may complement preformed vitamin A in the diet under certain conditions.

Already 40 years ago, several studies have suggested that beta-carotene exerts a function, independent of vitamin A in bovine reproduction (12). Cows fed supplemental beta-carotene exhibited a reduced interval to first estrus, increased conception rates and reduced frequency of follicular cysts compared to animals receiving only vitamin A. Later it was reported that the pregnancy rate was increased in heat-stressed cows supplemented with beta-carotene (11). One of the underlying factors could be that both corpus luteum and follicular fluid of the cow have a high concentration of beta-carotene (8) and that cattle have the capacity to convert beta-carotene to retinol in the ovary (13), where beta-carotene may act as a local supply of retinol. Highest cleavage activity was found in large, pre-ovulatory follicles (14). Furthermore, the concentration of vitamin A in follicular fluid was significantly influenced by follicle quality, with highest concentrations in non-atretic follicles and lowest values in greatly atretic follicles. These findings could be an indication that by influencing hormone and protein synthesis, vitamin A may have a potential for local modulation of follicular development and therefore be one of the factors controlling recruitment, selection and growth of the dominant follicle in cattle (15). The steroid hormone progesterone, which is important for the preparation of the uterus for implantation and also supports the early development of the embryo, might be another important factor. Plasma beta-carotene was found to be positively correlated to progesterone production by corpus luteum cells, indicating that beta-carotene status is related to bovine luteal function (16).

Vitamin E (alpha-Tocopherol)

Within a group of chemically related compounds (tocopherols and tocotrienols), possessing vitamin E activity, alpha-tocopherol represents the most potent form of vitamin E. Alpha-tocopherol is nature's most powerful lipid-soluble antioxidant and has been recognized as an essential nutrient for all species, including ruminants. It is required for body functions such as growth, reproduction or health and secures the integrity of important tissues of the reproductive, muscular, circulatory, nervous and immune systems. Animals are unable to synthesize vitamin E and therefore are dependent on dietary sources to fulfill their requirement. Vitamin E is widespread in nature with the richest sources being vegetable oils, oilseeds and grains, legumes, green plants and pastures. Since the most commonly used feed ingredients contain variable amounts of vitamin E and stability of naturally occurring topcopherols is poor, leading to substantial losses of activity in feedstuffs during processing and storage, dietary supplementation of vitamin E is necessary to secure the needs of high yielding beef and dairy cows.

Following ingestion tocopherol esters, which are stable forms used in animal feeds and supplements, are first hydrolyzed in the intestinal lumen and the free alcohol is absorbed together with other fats, supported by bile salts and pancreatic lipase (17). Subsequent to absorption in the upper two-thirds of the small intestine, vitamin E is transported to the liver and finally deposited in cell membranes of various tissues. By protecting the structural polyunsaturated phospholipids from oxidation, vitamin E maintains the integrity of the cell membrane. In this context, the trace element selenium must be mentioned as well. Selenium is a cofactor of the enzyme glutathione peroxidase (GSH-Px), which is an integral part of the antioxidant system, acting in aqueous intracellular and extracellular compartments and exerting synergistic effects to vitamin E.

When liver stores of alpha-tocopherol are exhausted, low vitamin E diets result into irreversible deficiency (18). Clinical vitamin E deficiency in cattle appears as nutritional muscular dystrophy, called white muscle disease, occurring predominantly in calves. Through muscular degeneration and general weakness of muscles this condition results in lameness or paralysis and may lead to death of the animal due to heart failure. Osmotic fragility of erythrocytes has also been reported to increase in some instances of vitamin E deficit. More frequently and growing in importance are subclinical deficiencies of vitamin E such as poor reproductive performance or low immune responsiveness, which remain usually unrecognizable, but do influence considerably the costs of milk and meat production. In order to enable ruminants to entirely express their genetic performance potential, an adequate supplementation of the diet with vitamin E is necessary.

Several studies have shown that prepartum administration of vitamin E, selenium or both in combination improved fertility of dairy cows. A probable explanation for that finding may be that the endocrine glands, particularly the pituitary have high vitamin E levels, when compared with other organs. Moreover, vitamin E is thought to promote the release of important steroid hormones, which are regulating the reproductive cycle (19). Oral vitamin E supplementation to cows during the dry period in combination with selenium injections reduced the incidence of retained placenta from 17.5% to 0% (20). An injection of vitamin E and selenium 3 weeks before expected parturition lowered incidence of retained fetal membranes, increased the percentage of cows pregnant to the first service, reduced the number of services per conception and lowered the interval from calving to conception (21). Feeding cows supplemental vitamin E for 42 days prepartum was shown to reduce days to first observed estrus, days to first artificial insemination and tended to reduce days open (22).

The antioxidant activity of vitamin E contributes also to the improvement of the immune response, observed in vitamin E supplemented animals. Free radicals are produced during the so-called respiratory burst, a process which is associated with bacterial killing subsequent to phagocytosis by activated immune cells. Excess of free radicals catalyzes lipid peroxidation, causing damage to cellular and subcellular structures. The rapidly proliferating cells of the stimulated immune and phagocytic systems are particularly prone to such oxidative damage by free radicals, peroxides and superoxides. Vitamin E, which is present in high concentrations in immune cells, such as neutrophils, macrophages and lymphocytes, can inhibit these processes by quenching the highly reactive, potentially harmful free radicals. Vitamin E also appears to be involved in the conversion of arachidonic acid into prostaglandins and leukotrienes. These compounds play an important regulatory role in any biological processes, including immunity. Results from a number of controlled studies in ruminants suggested that vitamin E supplementation could improve the protective effects of vaccination. Calves, supplemented with vitamin E developed higher antibody titers against bovine herpesvirus, which was applied by intranasal vaccination (23). Steers, supplied with selenium and/or vitamin E, showed higher serum antibody titers to Pasteurella haemolytica antigen subsequent to a respective vaccination, however performance and health status were not affected (24). In beef heifers, no effect of either vitamin E or selenium supplementation on the humoral response to a Brucella abortus vaccination could be observed. However, the supplementation of cattle with vitamin E did enhance the natural antibody titer to Salmonella typhimurium (25). Regarding cell-mediated immunity, lymphocyte responses to pokeweed mitogen were increased when the cells had been isolated from calves, supplemented with alpha-tocopherol and/or selenium (26). Supplemental vitamin E was shown to specifically stimulate phagocytosis of Staphylococcus aureus by bovine neutrophils (26). Since neutrophil function in dairy cattle is suppressed around the time of calving, high levels of vitamin E effectively prevented suppression of blood neutrophil and macrophage function in this critical period (28).

Bovine mastitis is the most costly disease affecting livestock in many countries of the world. Mastitis is an inflammation of the mammary gland, resulting from an infection by bacterial or mycotic pathogens. Control of bovine mastitis can be achieved by either decreasing the exposure of teat ends to pathogens or by increasing the natural resistance of the mammary gland to infection. Many studies have clearly demonstrated that dietary vitamin E and selenium are able to increase considerably the resistance of the dairy cow to this disease. When dry cows were supplemented with vitamin E, a significant reduction of clinical mastitis was observed and the duration of the disease was substantially reduced (29). First lactation heifers, supplemented with selenium and vitamin E had significantly fewer quarters infected at calving, fewer cases of clinical mastitis and lower milk somatic cell counts (30). Vitamin E levels in blood plasma and milk samples of healthy cows were significantly higher than the corresponding values of the mastitic cows and the lowest

vitamin E values were recorded in milk samples from the most inflamed quarters (31). Furthermore there was a negative correlation between vitamin E and milk somatic cell counts. In an extensive field study with nine well-managed dairy herds, bulk tank somatic cell counts and rate of clinical mastitis was negatively related to plasma selenium level and concentration of vitamin E (32). In another study with dairy cattle, supplementation of diets with vitamin E significantly lowered milk somatic cell count, but did not reduce the incidence of clinical mastitis (33). Neutrophil function, which represents a primary defense mechanism to bacterial infections, is depressed during the early postpartum period, when the risk of intra-mammary infection is highest. Elevated vitamin E supplementation prevented this suppression, resulting in no difference of neutrophil functions before and after parturition (34). Moreover, vitamin E supplementation significantly enhanced chemotactic responsiveness of neutrophils (28). In lactating cows, fed supplemental selenium and/or vitamin E, the ability of peripheral blood neutrophils to kill Staphylococcus aureus was improved. When E. coli was the target organism, the improvement in killing ability was greater for vitamin E than for selenium (35). These studies strongly suggest that dairy cow diets deficient in vitamin E and selenium result in increased incidence of mastitis.

Meat has a relatively short shelf life, since the free radical induced lipid oxidation in the muscular cell membranes results in the development of rancidity, which is the main cause of food spoilage. The occurrence of off-odors and off-flavors in rancid meat originates from a number of undesirable and potentially harmful breakdown products of polyunsaturated lipids. Vitamin E is nature's most powerful lipid-soluble antioxidant, being able to break the free radical induced chain reaction of lipid oxidation. Numerous studies with meat from cattle, fed on diets supplemented with elevated levels of vitamin E, have shown that the oxidative stability of lipids was improved and that the development of rancid deterioration of the meat was delayed (36). Vitamin E also slows down the oxidation of the color pigment myoglobin and thus stabilizes the visual appearance of the fresh meat during refrigerated storage (37). Furthermore, by improving the integrity of the cell membranes, vitamin E can reduce drip loss from the carcass (38), a process which is considered unacceptable by most consumers. Therefore supplementation of livestock animals with extra vitamin E is the most promising approach to achieve and maintain an optimum quality of beef meat. The ability of vitamin E to prevent lipid oxidation has also been taken to develop a strategy for improving the oxidative stability of dairy products. Problems with spontaneous oxidation of milk fat are well-known in the dairy industry worldwide. When feeding vitamin E, alpha-tocopherol content in milk fat was increased and thiobarbituric-acid-reactive substances (TBARS), being indicators of lipid oxidation, were lowered in comparison to unsupplemented controls (39). Supplementation of high vitamin E substantially increased alpha-tocopherol concentration in blood and milk, improved milk production and tended to reduce oxidized flavor (40). When fatty acid profiles of milk fat towards a higher degree of unsaturation were modulated by feeding oilseeds, a supplementation of cows with vitamin E prevented the milk fat from oxidation and allowed production of butter with high oleic acid content and consequently a good spreadability (41). Yet, high levels of dietary vitamin E were required for this application due to low rate of transfer of tocopherols into milk.

Vitamin D_3 (Cholecalciferol)

Vitamin D designates a group of closely related compounds that possess antirachitic activity. It may be supplied through the diet or can be produced endogenously by irradiation of 7-dehydrocholesterol in the skin. Dietary vitamin D_3 is absorbed from the intestinal tract in association with fats and requires the presence of bile salts for absorption. Thereafter it is transported with other neutral lipids via chylomicrons into the lymphatic system of animals and deposited in the liver, in adipose and in muscular tissues. The activation of cholecalciferol occurs over 2 subsequent hydroxylation steps. The first conversion takes place in the liver to produce 25-hydroxy-vitamin D3 [25-(OH)-D3] and the second in the kidneys to the hormonal form 1,25-dihydroxy-vitamin D3 [1,25-(OH)2-D3]. This metabolite represents the active form of vitamin D3 that regulates the calcium-phosphorous homeostasis in the blood and controls deposition of minerals in the bones. Moreover, there is increasing evidence, which suggests a regulatory role of vitamin D3 in immune cell functions.

With adequate direct exposure to sunlight, ruminants do not have an absolute dietary requirement for vitamin D, due to the endogenous production in the skin. But a significant proportion of dairy cattle are housed under confined conditions and therefore are dependent on a dietary supply of vitamin D3 in order to avoid the

occurrence of vitamin D deficiency. Rickets, the primary vitamin D deficiency disease, is a skeletal disorder of young, growing animals generally characterized by decreased concentration of calcium and phosphorus in the organic matrices of cartilage and bone. Symptoms of rickets lead to various skeletal changes such as weakened long bones, resulting in curvature and deformation, enlarged, painful hock and knee joints, general stiffness of gait, arched back and a tendency to drag hind legs. In the adult animal, osteomalacia is the counterpart of rickets. Cartilage growth in the adult has ceased and thus this condition is characterized by a decreased concentration of calcium and phosphorus in the bone matrix, resulting eventually in fractures and breaks. Moreover, the disturbance of calcium and phosphorus metabolism produces other symptoms such as reduced performance, hypocalcemia and reproductive failure. In deficient herds, calving rates are lower and calves can be born dead or weak. Hypocalcemia, either milk fever (parturient hypocalcemia) or unexplained lactational hypocalcemia and paresis, may also be observed as a result of chronic vitamin D deficiency in dairy cows.

Milk fever is a metabolic disease, characterized by hypocalcemia at or near parturition in dairy cows (42; 43). The origin of milk fever is a failure of calcium homeostasis in periods of increased metabolic demand for calcium such as at the initiation of lactation. Milk fever usually occurs shortly after parturition and is manifested by circulatory collapse, generalized paresis and eventually coma and death. The most obvious and consistent clinical sign is acute hypocalcemia and treatment with intravenous calcium is an extremely effective cure. Parturient paresis can be prevented effectively by feeding a low-calcium diet for the last several weeks prepartum, followed by a high-calcium diet after calving (43). In addition, feeding high doses of vitamin D for three to eight days prepartum prevented 80% of expected milk fever cases in cows (44). However, prolonging the treatment to 20 days prepartum has resulted in toxicity. Also the combination of 25-(OH)-D3 with 1alpha-hydroxycholecalciferol was demonstrated to reduce significantly the incidence of parturient paresis in dairy cows fed high dietary calcium (45). The most practical approach to controlling milk fever appears to be through optimizing macro-mineral levels in the diet, use of anionic diets and providing continuous supplementation with vitamin D at normal levels.

Consumption of excessive doses of vitamin D can result in toxicity, usually manifested by calcification of soft tissues. With normal dietary supplementation such problems should not occur, since short-term administration of as much as 100 times the requirement level can be tolerated by ruminants and the presumed maximum safe level of vitamin D3 for long-term feeding conditions is four to 10 times the dietary requirement. But grazing animals can develop calcinosis, a disease characterized by deposition of calcium salts in soft tissues, due to ingestion of leaves of certain plants such as Solanum malacoxylon or Trisetum flavescens. These plants contain a water-soluble glycoside of 1,25-(OH)2-D3, which results in a massive increase in the absorption of dietary calcium and phosphorus such that normal physiological processes are unable to compensate (46).

Vitamin D3 is the principal source of supplemental vitamin D for livestock diets. Respective product forms for the animal feed industry are usually spray-dried gelatin beadlets, which offer the greatest vitamin D3 stability. Recently, a new commercial product, based on 25-(OH)-D3, has been developed, which bypasses the metabolization step in the liver and thus represents a more potent form of vitamin D.

Water-soluble Vitamins

It is still disputed in the scientific community, whether ruminants have a requirement for dietary supplementation of water-soluble vitamins. As no deficiency symptoms of this vitamin group occur in ruminants, it was concluded that rumen microbes are able to synthesize adequate quantities of these vitamins from typical forages and feedstuffs. Recent research suggests however, that high-yielding beef and dairy cattle need dietary supplementation of certain B-vitamins in order to optimize their health and production performances. Special attention has been attributed to niacin, folic acid and vitamin B_{12} (47). For this summary, biotin has been selected as an example to further underline the importance of the whole vitamin B-group.

Biotin (Vitamin H)

Biotin is a water-soluble vitamin with a rather complex chemical structure of three asymmetric centers, allowing 8 different stereoisomeric conformations to occur. However, only the D-biotin isomer is biologically

active. Biochemically, biotin acts as a coenzyme of various carboxylases, which have central functions in the carbohydrate, fat and protein metabolism. More particularly, biotin has been shown to support gluconeogenesis, fatty acid chain elongation and protein synthesis. Accordingly, it is vital for both growth and health of all animals. Biotin is present in most feedstuffs, but the quantities are usually small, the levels are variable and not all biotin is biologically available. Until recently, ruminants have been considered to be self-sufficient for biotin as bacteria in both rumen and large intestine produce this vitamin endogenously.

Research on the potential benefits of biotin for the integrity of hoof horn started more than 20 years ago (48). The first investigations were performed in pigs and horses, where improvements of the hoof horn condition were observed with biotin supplementation. As the hooves of horses and ruminants are structurally similar, biotin supplementation was also considered as a dietary strategy to prevent lameness in dairy cattle, which in terms of importance ranks third after mastitis and fertility problems. Roughly 80% of the cases of lameness can be attributed to hoof disorders. In a series of controlled trials, dietary biotin was tested for its ability to improve hoof horn conditions in dairy cattle and accordingly to reduce disorders of the claw and subsequent lameness. In one study the claw horn in biotin-supplemented dairy cows was significantly harder and both incidence as well as severity of interdigital dermatitis, sole contusions, sole ulceration and heel erosion was reduced (49). In another study the prevalence of white line separation was lower for biotin-supplemented than for control cows (50). In a double-blind long-term experiment with 2700 dairy cows from 20 farms, the biotin supplemented animals showed better locomotion scores and reduced lameness in all hooves, while the unsupplemented cows required more antibiotic treatments (51).

Since biotin is efficacious to reduce hoof disorders, an increase in milk yield could be expected from the improved health status of the cows. In a controlled 12 months field study, claw conditions were improved and milk production was significantly greater in the biotin supplemented cows (52). When supplementing Holstein cows with biotin from 14 days prepartum until 100 days in lactation, milk and protein yields were linearly increased (53). Also in the clinical study, focusing on white line separation (50), milk production was significantly higher for the supplemented than for the control cows. Finally, using rumen-protected biotin, milk yield, fat content and total protein were found significantly improved (54). Based on these results, it was speculated whether biotin did not have a direct physiological effect on milk production, be it via improvement of gluconeogenesis or through acceleration of fatty acid synthesis.

Conclusions

It is widely accepted by the livestock industry that the minimum dietary vitamin levels, required to prevent clinical deficiencies may not support optimum health, performance and welfare of livestock. An adequate vitamin nutrition compensates for higher demand of modern animal genotypes, for additional benefits such as modulation of the immune response, resistance against infectious diseases and other disorders and ultimately improves the welfare of farmed animals. Therefore, the vitamin nutrition of ruminants deserves special attention in order to maximize productivity of a high-quality end-product in a sustainable manner.

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Functional aspects of supplementing selected water-soluble vitamins in ruminants

Funktionelle Aspekte der Zufuhr ausgewählter wasserlöslicher Vitamine beim Wiederkäuer

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Based on vitamin research in cattle conducted almost half a century ago mature ruminants are considered not to rely on the exogenous supply of water-soluble vitamins (B-vitamins, ascorbic acid), because these vitamins are synthesized either by the rumen microflora or endogenously in tissues such as the liver in amounts sufficient to prevent clinical signs of deficiency and to optimize performance and health. However, this general dogma might be put into question, because performance of milk and beef cattle has increased markedly over the last decades and several recent studies reported that supplementing specific water-soluble vitamins improves performance and/or health of dairy and beef cattle, which is exemplified herein for two B-vitamins (biotin, niacin) and ascorbic acid.

Rationale and functional aspects of supplementing biotin in dairy and beef cattle

Biotin is indispensable for intermediary metabolism of all animals because it is required as a cofactor of four carboxylases acting in gluconeogenesis (pyruvate carboxylase, propionyl-CoA carboxylase), lipogenesis (acetyl-CoA carboxylase) and amino acid catabolism (β-methylcrotonyl-CoA carboxylase). To ruminant species, biotin is available from both, ruminal synthesis and ruminal feedstuff (pastures, forages). In spite of this, several studies demonstrated that apparent ruminal synthesis (ARS) of biotin calculated as the difference between duodenal flow and intake from feed in dairy cows and growing cattle is very low (< 1 to 3.1 mg/day [1, 2]) or even negative (-1.4 to -10.9 mg/day [3, 4]). In addition, it is noteworthy that the ARS of biotin is largely independent of the forage-to-concentrate ratio, even being very low when the ration is appropriate for ruminants [4]. Based on the abovementioned studies, the duodenal flow of biotin can be considered as rather scarce and is likely not sufficient to optimize performance and health of high-producing cattle. In line with this, increasing duodenal low of biotin by supplementation of biotin (20-30 mg/cow) was found to improve the biotin status of dairy cows as evidenced from increased plasma, milk and urine concentrations of avidin-binding substances [5-8], and from elevated hepatic activity of pyruvate carboxylase [7]. Moreover, a meta-analysis, which included a total of 11 studies from different groups, demonstrated a positive effect of biotin supplementation on performance of dairy cows [9]; i.e., supplementation of biotin (20 mg/day and 0.96 mg/kg TM, resp.) increased milk yield and dry matter (DM) intake by 1.66 kg/day and 0.87 kg/day, respectively. Another meta-analysis, which included 6 studies, revealed a 1.30 kg/day-increase of milk yield by biotin supplementation in dairy cows [10]. The improved milk yield is, at least partially, explained by the increased DM intake and, thus, nutrient and energy intake. Probably, this also explains that biotin supplementation increased milk yield only in high-producing, but not in low-producing dairy cows [7], in which nutrient and energy intake is not limiting for milk production. In agreement with this, no improvement of milk yield by biotin supplementation was observed in dairy cows with low milk yield [11]. Additionally, indications from earlier in vitro-studies exist that improvements of fiber digestion and an increased ruminal production of propionate due to biotin supplementation might contribute to the increase of milk yield [12, 13]. Apart from an improvement of milk yield, the meta-analysis from Chen et al. (2011) revealed positive effects of biotin supplementation on milk fat yield and milk protein yield (each +0.05 kg/day). An increased rate of hepatic gluconeogenesis [7], which increases the supply of glucose to the mammary gland thereby stimulating production of NADPH from hexose monophosphate pathway for fatty acid synthesis, might account for the increase of milk fat yield [9]. The increased DM intake of biotin-supplemented dairy cows has been attributed to an improved hoof health and reduced occurrence of hoof diseases and lameness as demonstrated in a large number of studies with dairy and beef cattle [11, 14-18], because lame cows are more likely to lie down and show less desire to eat [5]. Improvements of hoof health in response to biotin supplementation (20 mg/day) are likely due to the involvement of biotin in two major pathways in keratinization of epidermal cells, keratin protein synthesis and lipogenesis, both of which are critical for proper horn quality [19]. In line with this, experimentally-induced biotin deficiency in calves was found to cause a dyskeratotic epidermis of poor quality and functional capacity which was associated with claw disease [19].

Rationale and functional aspects of supplementing niacin in dairy and beef cattle

Niacin which encompasses two compounds, nicotinic acid (pyridin-3-carbonic acid) and nicotineamide

(NAM), is required in all animal tissues as precursor of the coenzymes NAD^+ und $NADP^+$, both of which facilitate the electron transfer within a large number of redox reactions (approx. 200) in either katabolic (glycolysis, tricarbonic acid cycle, fatty acid oxidation) or anabolic metabolic pathways (lipogenesis, cholesterogenesis, steroid hormone synthesis). Niacin is provided to ruminants from various feed sources, ruminal synthesis and endogenous synthesis from tryptophan (approx. 1 mg per 60 mg tryptophan). The ARS of niacin is generally markedly higher when compared to other B-vitamins and most studies showed a significantly positive ARS of niacin (179-586 mg/day [1], 210 mg/day [20], 2171 mg/day [3], 446-1547 mg/day [4]). However, investigations from Schwab et al. (2006) demonstrated that the ARS of niacin is strongly dependent on the feeding ration composition; i.e., while the ARS of niacin is positively correlated with the intake of non-fiber carbohydrates (NFC) and starch as well as the starch content of the feeding ration, it is negatively correlated with the dietary NDF content. Likewise, a positive correlation was found between ARS of niacin and the amount of starch digested in the rumen [21]. The intake of OM-adjusted ARS of niacin was found to be between 25-44 and 73-79 mg/kg for rations with 30% and 40% NFC, respectively [4]. In a more recent study, ARS of niacin was reported to be positive in cows fed a ration based on orchard grass silage (380-519 mg/day), but negative in cows fed a ration based on alfalfa silage (-3168 bis -300 mg/day). Moreover, the abovementioned studies generally demonstrated that the ARS of niacin is low when the niacin intake is high. Accordingly, supplementation of high doses of unprotected niacin in dairy cows and beef cattle even caused a negative ARS of niacin [20, 22] indicating a strong ruminal degradation of niacin at high intake. In line with this, an almost complete degradation of unprotected supplemental niacin in dairy cows was reported from Santschi et al. [3]. In addition, Castagnino et al. [23] found a negative correlation between ARS of niacin and niacin intake in dairy cows.

Interestingly, studies in dairy cows in which the duodenal niacin flow was increased by either abomasal niacin administration or feeding of rumen-protected (RP) niacin clearly show that high niacin doses exert biological effects going beyond the classical coenzyme-dependent effects of niacin. For instance, several studies reported antilipolytic effects in dairy cows [24-28], which have been explained at least partially by inhibition of the niacin receptor HCAR2 in white adipose tissue (WAT). Such an effect would be expected to be beneficial with regard to prevention of typical postpartal metabolic disorders such as fatty liver and ketosis, because extensive mobilization of non-esterified fatty acids (NEFA) from WAT in connection with a low-grade systemic inflammation during early lactation are considered to play a key pathophysiological role in these disorders [29]. In fact, two recent studies with dairy cows demonstrated a decrease of liver fat content in response to feeding RP niacin (7.8-16 g niacin/day, [27, 28]). In addition, a decrease in the plasma concentration of the ketone body β-hydroxybutyrate (BHBA) was found in studies with dairy cows fed high doses of RP niacin [27, 30] and even unprotected niacin [31, 32]. However, in one of the studies with dairy cows reporting a decrease of plasma BHBA concentrations by supplemental niacin, no reduction of plasma NEFA concentrations was observed indicating that the antilipolytic effect of niacin alone cannot completely explain reduction of BHBA levels. It is therefore not unlikely that high doses of niacin exert direct effects on hepatic metabolism, especially because in cattle HCAR2 is abundantly expressed not only in WAT, but also in parenchymal cells of the liver [33]. In line with this, we found that supplementation of RP niacin causes profound changes in the hepatic transcriptome of early-lactation dairy cows [34]. Of note, the genes identified to be up-regulated in cattle liver by RP niacin were almost exclusively involved in physiological processes dealing with immune function. This might explain observations from other studies that high niacin doses in dairy cows (24 g/day) modulate different functions of immune cells such as phagocytosis, stress response and even apoptosis [35-37]. A further interesting functional effect of high doses of supplemental RP niacin (12 g/day) in cattle is an increased resistance to thermal stress as evident from an increment of evaporative heat loss through increased sweating rate and a decrease of body temperature [38, 39]. This effect has been attributed to the induction of skin flushing by nicotinic acid [40], thereby, increasing peripheral heat loss [41]. Skin flushing is mediated by activation of HCAR2 receptor in Langerhans cells of the skin, which induces prostaglandin D (PGD) release leading to PGD receptor-mediated vasodilatation of blood vessels in the skin [42]. A further mechanism of supplemental RP niacin in alleviating heat stress may involve a dramatic increase of water intake in dairy cows under conditions of thermal stress [43].

Despite the induction of specific metabolic and immunologic effects, supplemental niacin appears to have little effect on milk performance; i.e., a large number of studies demonstrated no influence of high niacin

doses on milk yield independent of the presence or absence of rumen-protection [27, 28, 30, 31, 43-48]. Only very few studies reported a milk yield-increasing effect of supplemental niacin [49-51]. In contrast, supplementation of RP niacin in dairy cows consistently decreased milk fat concentration and milk fat yield [27, 28, 30], - effects that might be partially explained by the abovementioned antilipolytic effect of supplemental niacin [28].

Rationale and functional aspects of supplementing ascorbic acid in dairy and beef cattle

L-ascorbic acid, also known as vitamin C, is not an essential nutrient in adult ruminants like in other farm animals, because it is synthesized in sufficient amounts in the liver from glucose via the glucuronic acid pathway [52]. However, proper functioning of endogenous synthesis is more important for ruminants than for monogastric farm animals, because vitamin C from alimentary sources is strongly degraded in the rumen [53]. According to [54], the half-life of ascorbic acid in the rumen is only 3.5 h. In contrast to adult ruminants, calves up to an age of 2-3 weeks rely on ascorbic acid from either prenatal storages or intake from colostrum, mature milk or an appropriate milk replacer, because hepatic synthesis is not sufficiently developed during early postnatal life reaching its maximum only after 8-16 weeks of life [55, 56]. This probably explains the occurrence of scurvy-like dermatoses in newborn calves at low vitamin C supply from the diet, which is successfully treated with injections of ascorbic acid [57].

Despite endogenous synthesis of ascorbic acid in adult cattle, several indications exist that endogenous synthesis in the liver is not sufficient to meet the demand under different conditions of exogenous and endogenous stress (e.g. infectious diseases). For instance, plasma concentrations of vitamin C were reduced in lactating cows exposed to heat stress [58]. In addition, cows with experimentally-induced (E. coli infusion) mastitis had decreased concentrations of vitamin C in plasma and milk [59-61]. Moreover, the severity of clinical mastitis symptoms was found to be negatively correlated with vitamin C concentration in plasma and milk [59]. Interestingly, the plasma vitamin C concentration was also reduced in lactating cows exhibiting signs of hepatic malfunction or hepatic damage, such as decreased plasma concentrations of albumin and total cholesterol and increased plasma activities of hepatic enzymes (alkaline phosphatase, aspartate aminotransferase) [62]. The authors of the latter study suggested that impaired hepatic ascorbic acid synthesis due to hepatic malfunction, which is frequently found in high-yielding dairy cows during early lactation, accounts for the reduced plasma ascorbic acid concentrations of such cows [62]. In line with this, decreased plasma concentrations of ascorbic acid were also demonstrated in early lactation dairy cows with ketosis compared with cows without ketosis [63], even though this difference lacked statistical significance.

In addition to the abovementioned findings, several studies in cattle and other ruminants showed that parenteral vitamin C administration or oral administration of RP forms of vitamin C (ascorbyl-phosphate, ascorbic acid coated with ethylcellulose, silicone or hydrogenates soybean oil), which show a high bioavailability [63-67], is of prophylactic or therapeutic value. For instance, supplementation of ascorbyl-phosphate alleviated the effects of heat stress in buffalo cattle [68]. Supplementation of high doses of vitamin C reduced transport stress-induced hemolysis in goats kept under dry-hot conditions [69, 70]. Moreover, parenteral vitamin C-injection alone or in combination with antibiotic therapy had a therapeutic effect in cattle with mastitis [61, 71, 72]. Furthermore, another study demonstrated a reduction of milk somatic cell count due to feeding 30 g ascorbyl-polyphosphate to cows injected with LPS into the mammary gland [73]. In beef cattle, several studies from Pogge et al. [74-76] revealed that supplementation of ascorbic acid beneficially influences different carcass characteristics and parameters of meat quality, such as marbling score and oxidative stability.

Conclusion

Recent studies in cattle, particularly dairy cattle, demonstrate that supplementation of high doses of selected water-soluble vitamins in RP form provides a strategy to improve health (ascorbic acid, biotin), to alleviate the effects of different kinds of stress (niacin) and to even increase production parameters (biotin). Thus, the general dogma that mature ruminants do not to rely on the exogenous supply of water-soluble vitamins should be considered as outdated.

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Preis: (D) € 29,90

ISBN: 978-3-7690-4112-5