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W. Windisch
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Nutrition of veal calves: Interactions between Milk Replacer and Solid Feeds

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Introduction: Solid feeds (SF), comprising roughages and concentrates, represent an increasingly important source of nutrients for veal calves. From a welfare and economic perspective, there is a strong incentive to replace a considerable portion of the milk replacer (MR) by SF in the diet (see e.g. (1)). Interactions between MR and SF, mostly occurring in the gastro-intestinal tract, complicate the prediction of the nutritional value of these ration components. These interactions already start at the onset of rumen development and include recycling of urea originating from amino acids from the MR, ruminal milk, influences of SF on passage rate kinetics of the MR or vice versa. In older calves, glucose homeostasis has been long known to be a challenge for veal calves fed considerable quantities of lactose or other carbohydrates via the MR. This abstract describes findings, largely obtained at Wageningen University, illustrating interactions of MR and SF components in veal calves.

Rumen development: Rumen development is initiated upon the consumption of solid feeds, triggering the production of short-chain fatty acids. The importance of the composition of the solid feed, in particular the presence of course particles via the roughage portion of the SF fraction has been recently reviewed by Khan et al. (2) and the importance of a well-developed rumen for the development of the lower gut by Steele et al. (3). Even though the MR is assumed to be directed towards the abomasum, thus bypassing the rumen upon closure of the oesophageal groove, the MR may influence rumen development in various ways. Increased MR intake has been demonstrated to reduce concentrate intake (4, 5). After 5-6 weeks of age, substantial increases in MR from 0.6 kg DM/d in week 6 to 1.4 kg/DM/d in week 10 levels substantially reduces SF intake (6). Whether increases in the roughage portion of the SF component depresses DM intake is a matter of debate (see (2), but the effect seems quite limited at lower roughage proportions. The roughage portion of the SF is important for calves in many ways (2). It is important for rumination behaviour, reducing abnormal oral behaviours (1). The intake level of concentrates, rather than its composition, stimulates the production of short-chain fatty acids. Remarkably, a rumen pH of around 5.0 can be observed in calves fed concentrates and MR, without obvious detrimental health effects and maintaining calf performance (4). Apparently, the nutrients absorbed from the MR effectively buffer the acids absorbed from the rumen and no signs of acidosis can be observed from the outside of these calves. Rumen development, however, is negatively affected under such conditions, with typical branching and lumping of rumen papillae (2, 7), and formation of plaques on the rumen wall (7). It cannot be excluded that components of the MR, via ruminal drinking, contribute to these plaques. Although quite normal concentrations of short-chain fatty acids are observed with compared with dairy cattle, the presence of substantial quantities of reducing sugars in the rumen contents suggests hampered uptake of glucose by rumen microbes under such conditions (4). It was demonstrated that inclusion of 30% of roughage in the SF is sufficient to prevent formation of plaques on the rumen wall (6).

Urea recycling: Recycling of urea has been demonstrated and measured in ruminants (8). It is commonly assumed to be triggered by low N concentrations in the rumen, but it cannot be excluded that the availability of urea in the circulation affects urea recycling. The influence of dietary crude protein content on urea recycling has been recently reviewed in cattle (9). In veal calves, typically showing low efficiency of conversion of absorbed amino acids to tissue deposition (10), urea production is high and largely originating from MR amino acids. In a study combining MR with low-protein SF, Berends et al. (11) demonstrated, using a [$^{15N}_{2}$]urea approach, that for every incremental g of DM from SF intake, nitrogen intake increased by 0.70 g, and nitrogen retention increased by 0.55 g ($P < 0.01$). Of this increase in nitrogen retention, 19% could be directly explained by urea recycling, with the remaining part being explained by increased intake of N via the SF, and an increased N efficiency related to increased ME intake. In a follow-up study, it was demonstrated that the contribution of urea recycling to the nitrogen economy of veal calves was substantially lower when a high-protein concentrate was fed (12). It was concluded that low N availability in the rumen limits microbial growth and rumen fermentation in calves fed low-N SF (93 g of CP/kg of DM), and this effect cannot be compensated for by recycling of urea originating from MR.

Feeding value of SF in veal calves: Evaluation of the feeding value of SF in veal calves is complicated by the simultaneous provision of MR. At the level of digestion, it is difficult to separate the contribution of the MR to ileal or faecal excretion. When feeding incremental quantities of SF at a fixed level of feeding

of MR, Berends et al. (13) demonstrated the apparent total tract digestibility of a SF mixture of concentrates, straw and corn silage (50:25:25 on a DM basis) to be 64 and 60% for DM and energy in calves in a BW range of 108 to 164 kg. Notably, the total tract digestibility of NDF increased significantly with BW from 46 at 108 kg BW to 56% at 164 kg BW. This increase in digestion efficiency with age or body weight was later also demonstrated by Berends et al (14). The cause of this age effect is not clear, but may be related to development of the motility of the gastro-intestinal tract with age. There is little information on the passage rate behaviour of SF components through the gastro-intestinal tract. In a study applying a single dose of different indigestible markers for concentrates (C36 alkanes), roughages (Cr-mordant long straw) and MR (Co-EDTA), Berends et al. (15) deduced fractional passage rates to be quite low when compared with other ruminants. Estimates for the fractional passage rate for concentrates were low (3.3 for low and 4.9%/h for high SF intake) and even lower for roughage (1.3 and 1.7%/h, also increased by the level of SF feeding). In addition, a reduced recovery of Co in the abomasum at slaughter in calves fed high levels of SF was observed, but was not considered as an indicator of altered passage kinetics of the MR. The ratio between Cr and C36 was similar in the small and large intestine, indicating that differences in the passage of concentrate and straw observed in this study were mainly determined by differences in rumen and abomasum emptying. When exchanging substantial quantities of MR for SF components, the experimental design used to estimate the feeding value for these ration components becomes critical. An incremental approach can be used to evaluate the effects of increments of SF intake at a fixed level of intake of the MR (see (16)). When the studies are conducted over a large age or weight range of the calves, differences in BW between treatments may become problematic. Attempts to exchange SF for MR, maintaining energy (or protein) intake are problematic when the digestibility, metabolizability or net energy values are not known. Quite often these are the subject of study. To this end, we used a paired-gain approach to study the feeding value of two mixtures of SF with a roughage:concentrate ratio of 50:50 or 20:80 on a DM basis (17). Dry matter (DM) intake from SF was targeted to reach 20, 100, 180, and 260 kg of DM for four SF levels, respectively, during the 16-wk experimental period, and increased with preplanned, equal weekly increments. The quantity of MR provided was adjusted every 2 wk based on BW to achieve similar targeted rates of carcass gain across treatments. The reduction in MR provided (in kg of DM) to realize equal rates of carcass gain with inclusion of SF (in kg of DM) was considered to represent the feeding value of the SF mixture relative to MR. After accounting for slight, unintentional changes of carcass gain with SF intake, the feeding value of the 20:80 SF mixture was found to be 10% higher compared with that of the 50:50 SF mixture. The feeding value of SF relative to that of MR also increased substantially with age, implying that additivity in feeding values of these ration components cannot be assumed.

Glucose homeostasis: Problems maintaining glucose homeostasis in heavy veal calves were already demonstrated in the previous century (18-20), influenced by for example age, the level of feeding of the MR and feeding frequency (18, 21), nutrient synchrony (22) and protein intake (23). These problems are characterized by hyperglycaemia, hyperinsulinemia and glucosuria. It is connected to the low priority of calves to channel glucose originating from the MR into *de novo fatty acid synthesis* (24), and to a low insulin sensitivity (19). Although clearly, postprandial glucose and insulin kinetics are influenced by MR intake, there is accumulating evidence indicating that insulin sensitivity is not substantially altered by the plane of MR feeding. MacPherson et al. (25) found no effect of plane of MR feeding on glucose an insulin responses to an intravenous glucose tolerance test. Yunta et al., (5) observed a tendency for a decrease in insulin sensitivity with increasing MR intake, particularly at 42 d of age. It should be noted that the intravenous glucose tolerance test was performed only after a 5 h fasting period. Recently, it was demonstrated that in calves, insulin sensitivity decreases rapidly, by almost 70%, between week 3 and 6 of age, unaffected by weaning (26). It seems therefore that insulin sensitivity in calves is quite high at birth, decreases rather independently of feeding strategy in early age to very low levels when compared with other species. In heavy calves, insulin sensitivity is invariably low, being rather insensitive to the carbohydrate source in the MR (27) or to an exchange of lactose for fat (euglycemic–hyperinsulinemic clamp, 28). Variation in postprandial glucose and insulin responses between dietary treatments (see (21, 22) are therefore unlikely to be caused by differences in insulin sensitivity. Little known about the effect of SF intake on insulin sensitivity in calves fed MR. Unpublished results from our lab indicate that incremental intake of SF reduce insulin sensitivity in veal calves, but all within the rather narrow and low range of insulin sensitivities commonly found in calves exceeding 100 kg of BW.

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1.

Early prepartum classification of high versus normal body condition in dairy cows leads to pronounced metabolic differences post partum

Frühzeitige präpartale Einteilung von Milchkühen anhand der Körperkondition, führt zu erhöhten metabolischen Unterschieden post partum

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For dairy cows overconditioning before calving is well known to result in excessive lipolysis with elevated blood concentrations of free fatty acids (NEFA) and ketone bodies and thus an increased risk for metabolic diseases. For experimental purposes feeding above energy requirements during the dry period is mostly used to induce overcondition, but did not always result in significant elevations of body condition or circulating NEFA concentrations, respectively [1-2]. The success of such elevated feeding regimen to cause overconditioning seems to be more probable if cows were preselected according to their body condition at drying off [2-3], thus indicating a genetic or epigenetic component for accumulating body fat. With this background we aimed to group dairy cows according to their body condition well before the dry period, i.e. during late lactation, and to feed them for enhancing or maintaining their high or normal body condition score (BCS) when still lactating, respectively, but not when dry, to test whether the difference would be sustained into lactation.

Methods: Fifteen weeks (wk) before the anticipated calving, 38 multiparous, pregnant Holstein Frisian cows were allocated in either a high (HBCS) or normal (NBCS) BCS-group according to their actual BCS and back fat thickness (BFT); while pre-selected for their history (previous lactation) of body condition. From then on, HBCS and NBCS cows were fed with either a high energy diet (7.1 NEL MJ/kg DM) or a low energy diet (6.6 NEL MJ/kg DM), respectively, until dry off. Thereafter, all cows received the same ration. Individual dry matter intake (DMI) was recorded with an electronic feeding system from d 21 ante partum (ap) until d 100 postpartum (pp), while feed was always offered ad libitum. During the entire study, BCS and BFT were obtained biweekly, while body weight (BW) before calving was recorded weekly. After calving, milk yield and BW were recorded daily; milk composition was analyzed weekly. In addition, weekly blood samples were collected from d 49 ap to d 84 pp. The serum concentrations of NEFA, ß-hydroxybutyrate (BHB), and glucose were quantified using an automatic analyzing system, based on a photometric measurement (Eurolyser, Type VET CCA, 5020 Salzburg, Austria). The data were analyzed by the mixed model procedure (SPSS, version 21.0) with Bonferroni correction. Calculated correlations were made using Pearson. Level of significance was set at $p \leq 0.05$. The data are presented as mean ± SE.

Results: Early allocation of cows to either a HBCS or a NBCS group led to significant differences in BCS and BFT ($p < 0.001$), that were maintained over the whole observation period. Considerable losses of BCS and BFT ($p \leq 0.001$) were observed in HBCS (BFT: 11 ± 0.9 mm) cows 7 wk after calving compared to NBCS cows (BFT 5 ± 0.8 mm). Accordingly, energy balance (EB) was more negative in HBCS cows ($p < 0.05$) several wk around calving. Contrastingly, DMI was higher in NBCS cows ($p < 0.05$) 3 wk before and 2-4 wk after calving. The serum concentrations of NEFA were increased in HBCS ($p < 0.05$) in the first six wks after calving. Additionally, BHB concentrations were greater ($p < 0.05$) in HBCS cows from 2 to 5 wk pp, peaking in wk 3 (HBCS: 1.6 ± 0.8 mmol/L; NBCS: 1.1 ± 0.5 mmol/L). Thereby, BHB increased more than 98 % for HBCS cows compared to wk 1 ap. The EB and NEFA values were inversely related ($p = 0.001$; R^2=-0.30), and NEFA and BHB were positively associated ($p < 0.001$; R^2=0.30). Similarly, serum glucose concentrations were positively correlated with BCS and BFT. However, milk yield, energy-corrected milk and milk components showed only weak differences between the groups.

Conclusion: Differences in BCS evolving during lactation and boosted by feeding in late lactation were shown to be maintained until the subsequent lactation, even in absence of differential feeding during the dry period. Group differences were confirmed by NEFA and BHB concentrations. Thus, allocating pre-selected late lactation-cows to a high or a low energy diet before dry off, is an appropriate approach to achieve target BCS at calving.

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2.

Effects of elevated BHB concentrations on glucose metabolism in dairy cows before and after parturition

Auswirkungen von erhöhten BHB-Konzentrationen auf den Glukosestoffwechsel von Milchkühen vor und nach der Abkalbung

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Recent studies in mid- and late lactating dairy cows showed that beta-hydroxybutyrate (BHB) infusion had considerable impact on glucose metabolism, and immune response during intramammary lipopolysaccharide challenge. The objective of the present study was to infuse BHB during the dry period and after parturition to investigate the effects of elevated plasma BHB concentrations on metabolism and endocrine changes in transition dairy cows. The hypothesis tested was that regulation of glucose metabolism changes at different physiological stages and an additional elevation of BHB concentration alters glucose concentration.

Methods: Multiparous Holstein cows in wk -2 (a.p.; n = 6) and wk +2 (p.p.; n = 8) relative to calving were infused (4 h from 0800 to 1200 h) with a BHB solution to increase plasma BHB concentration to 1.5 to 2.0 mmol/L (HyperB), and the same period on the next day without any infusion was assigned as control treatment (CON). Blood samples were taken 1 h before the start of infusion as reference samples and every 30 min during the following 6 h (4 h infusion and 2 h after the stop of infusion) in HyperB and on the control day, and analyzed for glucose, BHB, insulin, and glucagon concentrations. Statistical analysis was performed with SAS (Version 9.4, SAS Institute Inc., Cary, NC, USA). Differences in basal concentrations of plasma metabolites and endocrine parameters between a.p. and p.p., and between control and infusion d in wk -2 and +2 relative to calving were evaluated using the MIXED procedure of SAS with time points (a.p., p.p.) and paired experimental days (HyperB, CON) as fixed effects. The individual cow was used as repeated subject in the statistical model.

Results: Plasma BHB concentration reached 1.7 ± 0.1 mmol/L (a.p.), and 1.6 ± 0.2 mmol/L (p.p.) in HyperB compared with 0.6 ± 0.1 mmol/L, and 0.6 ± 0.0 mmol/L in CON, respectively. The 4-h average BHB infusion rate was 12.4 ± 1.0 and 13.3 ± 0.9 µmol/kg BW/min in wk -2 and +2, respectively. BHB infusion caused a decrease of plasma glucose concentrations compared with pre-infusion levels both before and after parturition, which was not different between a.p. and p.p. infusion though basal glucose concentrations were different before and after calving (3.7 ± 0.1 vs. 3.2 ± 0.2 mmol/L). BHB infusion increased plasma insulin a.p. but not p.p. despite higher basal insulin concentration before than after parturition (29.0 ± 8.4 vs. 5.8 ± 0.8 µU/mL).

Conclusions: These findings show that effects of hyperketonemia on plasma glucose concentrations are independent of lactational stage, but endocrine adaptation to hyperketonemia differs before and after parturition. We assume that BHB is a metabolic key regulator in early lactating dairy cows, and may affect glucose concentration by further pathways beyond regulation via insulin and glucagon, such as gluconeogenesis and altered lipolysis.

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3.

Effects of intensive milk feeding and butyrate supplementation on the somatotropic axis in German Holstein calves

Einfluss einer intensiven Milchfütterung und einer Buttersäurezusatz auf die somatotrope Achse bei Kälbern der Rasse Deutsche Holstein

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In traditional feeding programmes calves are reared with restricted amounts of milk or milk replacer (MR), not able to exploit their full growth potential (1). Intensified colostrum and milk feeding maturates the somatotropic axis indicated by a reduced growth hormone/insulin-like growth factor (IGF)-I ratio in blood plasma and an elevated IGF-I gene expression in the liver (2). In addition, butyrate supplementation in calves induces accelerated feed efficiency and growth (3). Therefore we hypothesised that the combining effect of intensive MR feeding and butyrate supplementation stimulates energy metabolism and the somatotropic axis in preweaning calves.

Methods: 64 German Holstein calves (n=32 for male and female, respectively) were studied between June 2014 and June 2015 from birth until wk 11 of life. Calves were allocated to one of four feeding groups after measurement of birth weight and colostrum intake (2.5 kg from their dam). Subsequent feeding with transition milk from their dams was supplied *ad libitum* (Adlib; max. 25 L/d, n=32) or in restricted amounts (Res; 6 L/d, n=32) until d 4. Afterwards Adlib and Res groups were subdivided (n = 16/group) to MR feeding at 12.5 % dry matter with (ResB+; AdlibB+) or without 0.24 % Ca-/Na-butyrate (ResB-; AdlibB-) from d 4 on. Gradually weaning took place from wk 9 to 10, whereas 2 L/d of MR were offered until the end of trial. Calves were housed in an open straw-bedded stable with an automatic feeding system and had free access to water, hay and concentrate. Measurements of feed intake were performed daily and body weight was determined weekly. Blood samples for analysing IGF-I, IGF binding proteins (IGFBP), insulin and glucose were taken after birth, on d 2, 4 and 7, then weekly or biweekly (IGFBP) until wk 11 of life. Liver samples were taken on d 50 ± 2 (mean ± SD) and at the end of the study to measure gene expression of the somatotropic axis. Data were analysed by the Mixed Model of SAS with feeding regimen, butyrate supplementation, time, and respective interactions as fixed effects.

Results: Except for the first colostrum intake (2.5 ± 0.09 kg) liquid feed consumption was much greater in Adlib than in Res groups ($P < 0.001$). Res had a greater concentrate intake ($P < 0.001$), but lower weight gain ($P < 0.001$) throughout the trial. Plasma concentrations of IGF-I, IGFBP-3, insulin and glucose were greater ($P < 0.01$) and plasma concentration of IGFBP-2 was lower ($P < 0.05$) in Adlib than in Res. Butyrate supplementation depressed ($P < 0.05$) plasma IGF-I from wk 1 - 4 and 9. On d 50, abundance of the hepatic form of the growth hormone receptor and of IGF-I was greater ($P < 0.01$) and mRNA abundance of IGFBP-2 was lower in Adlib than in Res. At the end of the study, IGFBP-2 mRNA abundance was greater in Adlib than in Res. Butyrate increased hepatic IGFBP-2 mRNA abundance at the end of the study.

Conclusions: *Ad libitum* MR feeding stimulated the systemic and hepatic somatotropic axis, which mirrored the greater growth rate during the intensive MR feeding. Butyrate supplementation did not stimulate growth performance but partly depressed the IGF/IGFBP system.

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4.

Organ and epithelial growth in the gastrointestinal tract of Holstein calves fed milk replacer *ad libitum* and supplemented with butyrate

Organ- und Epithelwachstum im gastrointestinalen Trakt bei Kälbern der Rasse Holstein mit ad libitum Milchfütterung und Butyratzusatz

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Previous studies have shown that an elevated milk feeding intensity during the first weeks of life play an important role for growth and development in preweaning calves (1,2). Furthermore, butyric acid (BA) is known to stimulate intestinal cell growth and maturation, especially in the small intestine (3). We hypothesized that a combination of intensive milk replacer (MR) feeding and BA supplementation expedites the development of the gastrointestinal tract as well as organ growth in preweaning calves.

Methods: German Holstein male calves (n=32) were studied from birth until d 81 (±2) of life. All calves received 2.5 kg colostrum during 2 hours after birth from their dams. For the first three days of life transition milk was offered twice a day and from day 4 onwards calves were fed MR (12.5 % dry matter) in amounts of either 6 l/d (Res; n=16) or *ad libitum* (Adlib; max. 25l/d; n=16) for 8 wk. In both feeding groups half of the calves (n=8/group) were fed MR with 0.24% Ca-/Na-BA (ResB+; AdlibB+) or same MR with no BA supplement (ResB-; AdlibB-). From wk 8 to wk 11 MR was linearly reduced in all calves to 2 kg MR/d. Hay, water and concentrate were offered *ad libitum*. At the end of the trial calves were slaughtered and liver, pancreas and kidney fat were weighted. The forestomaches were emptied, washed and weighted and the length of the small and large intestine was determined. Mucosa samples of the rumen (atrium, ventral sac, ventral blind sac) and small intestine (duodenum, proximal, middle and distal jejunum, ileum) were taken for histomorphometric measurement of rumen papilla and intestinal villus and crypt size. Data were analyzed by the Mixed Model of SAS with feeding regimen, BA supplementation, rumen/intestinal segment, and respective interactions as fixed effects and calf as random effect.

Results: Body weight at slaughter and kidney fat weight were greater ($P < 0.05$) but pancreas weight was lower ($P < 0.01$) in Adlib than in Res. The small intestine was 3.8 m longer ($P < 0.05$) in AdlibB- than in ResB-. No treatment differences were found for rumen papilla size, but villus circumference, surface and height in duodenum, proximal jejunum and ileum were greater ($P < 0.01$) in Adlib than in Res. Villus circumference, surface and height was greater ($P < 0.05$), except in duodenum, in B+ than in B-. Crypt depth was larger ($P < 0.05$) in Adlib than in Res in duodenum and proximal jejunum and was reduced ($P < 0.02$) by BA in ileum. The villus height/crypt depth ratio increased by *ad libitum* feeding and by BA and was greatest ($P < 0.05$) in AdlibB+ throughout the small intestine.

Conclusions: Intensive milk feeding increased body weight and small intestinal growth, whereas BA, partly together with intensive milk feeding, stimulated intestinal mucosal growth. These findings indicated an elevated maturation and absorbing capacity of the small intestine by intensive milk feeding and BA supplementation. Increased pancreas weight in Res calves may have compensated for insufficient nutrient supply. Ruminal mucosa was not affected by milk feeding regimen suggesting that intensive milk feeding did not impair rumen development.

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5.

Transcript abundance of lipolysis, lipid oxidation, lipogenesis and lipid transport genes in the skeletal muscle of normal and low birth weight pigs

Transkriptmengen von mit Lipolyse, Lipidoxidation, Lipogenese und Lipidtransport assoziierten Gene in Skelettmuskeln von Schweinen mit niedrigem und normalem Geburtsgewicht

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Skeletal muscle is a major site of carbohydrate and fat oxidation and the size and composition of porcine skeletal muscle is influenced by birthweight. Low birthweight pigs have smaller muscles and a different muscle fiber type composition that is associated with increased muscle hypertrophy and altered carbohydrate / fatty acid metabolism leading to impaired carcass traits (1). Previous work from our group has shown that between age d76 and d125 low birthweight (LBW) pigs have lower whole body fat oxidation and higher carbohydrate oxidation compared to normal birthweight (NBW) pigs underlying greater lipid deposition in LBW pigs (2). Thus, in this study we measured the mRNA abundance of genes involved in lipolysis, lipid oxidation, lipogenesis, de novo fatty acid synthesis and lipid transport in the longissimus dorsi (LD) and semitendinosus (ST) muscles of low and normal birthweight pigs, to determine if the previously observed changes in whole body fat and carbohydrate oxidation were related to altered skeletal muscle metabolism.

Methods: Female German Landrace LBW (0.8-1.2 kg) and NBW (1.3-1.8 kg) pigs were slaughtered at age d76 (n=20), d97 (n=18), d104 (n=18) and d131 (n=16). Semitendinosus (ST) and longissimus dorsi (LD) muscle samples were collected, cut into small pieces and snap frozen in liquid nitrogen. Total RNA was extracted from -80oC frozen ST and LD tissue, purified, quantified and quality assessed so that only samples with a RIN $\geq$ 8.5 were used for subsequent cDNA synthesis. DELTAgene primer assays were designed to amplify cDNA templates involved in the biological processes of lipolysis (n=7), lipid oxidation (n=5), lipogenesis (n=10), de novo fatty acid synthesis (n=3), lipid transport (n=2) and potential references templates (n=5). Real time PCR data quality was checked using the Fluidigm Real-Time PCR analysis software, balanced for reference gene selection using qBASEplus 2.0. Differences in mRNA abundance between birthweight groups at each timepoint (BWC) and between time points within birthweight group (TPC) were assessed using SAS 9.4 (Kruskal-Wallis PROC NPAR1WAY).

Results (BWC): At age d76 the mRNA abundance of nuclear receptor subfamily 4 group A member 1 (NR4A1), a key regulator of lipolysis, was lower in the LD of LBW compared to NBW piglets. Whilst at age d97 a significant increase in the mRNA abundance of genes involved in de novo lipogenesis (acetyl-CoA carboxylase alpha, diacylglycerol O-acyltransferase 1, peroxisome proliferator-activated receptor alpha (PPARa)) was observed in the LD of LBW compared to NBW pigs. No significant changes were observed at ages d104 and 131. In the ST muscle of LBW pigs at age d76 hormone sensitive lipase, and at d97 fatty acid synthase were higher compared to NBW. At age d104 the mRNA abundance of NR4A1 was higher and carnitine palmitoyltransferase 1a (CPT1a) and fatty acid binding protein 4 was lower in LBW compared to NBW pigs, indicating decreased lipid oxidation in the ST of LBW pigs. Whilst at age d131 adipose triglyceride lipase (ATGL) and PPARa was lower suggesting reduced lipolysis in LBW compared to NBW pigs. **(TPC):** The mRNA abundance of NR4A1, FASN and peroxisome proliferator-activated receptor gamma coactivator 1-alpha decreased in the ST of NBW from age d76 to 131, but not LBW. The mRNA abundance of ATGL and monoglyceride lipase decreased in the ST of LBW pigs but not NBW, from age d76 to 131.

Conclusions Taken together the data from this study suggests that the LD and ST muscles of LBW and NBW piglets regulate lipolysis, lipid oxidation, lipogenesis, de novo fatty acid synthesis and lipid transport via different mechanisms when switching from fatty acid to carbohydrate oxidation.

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6.

Data on energy and protein metabolism in growing cats - measured by indirect calorimetry

Daten zum Energie und Proteinstoffwechsel bei wachsenden Katzen - gemessen mit indirekter Kalorimetrie

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Literature data concerning energy and protein requirements of growing cats are rare. For this reasons we hypothesize that from data measured with help of indirect calorimetry more accurate data on protein- and fat deposition as well as energy requirements in growing cats can be calculated compared to those used as base for energy requirements for growth by the NRC (2006).

Methods: 27 kittens (12 females, 15 males) from the breeding family of the Institute of Animal Nutrition at the Vetsuisse Faculty, University of Zurich with a mean birth weight of 101.0g (SE ± 2.5) were investigated. All kittens of one litter lived together with their mothers until 9 weeks of age. Additionally, the kittens were fed with a moist commercial dry as well as mushed wet food ad libitum from the age of 3 weeks. The experimental dry food was fed from the 17th week of age until 8 months (mo) (crude protein: 34%, crude fat: 18%, crude fiber: 2%, crude ash: 7%), ad libitum. The kittens were weighed once a week until 8 mo of age. Body Condition Score (BCS) was evaluated monthly from 2 to 8 mo using a 9 point scale system (Laflamme, 1997). Dual energy X-ray absorptiometry was performed at 4, 6 and 8 mo. At 4 and 6 mo, energy expenditure was measured using indirect calorimetry. Each kitten was measured for 4 days, after an adaptation day. During the measuring periods, volumes of O2, CO2 and CH4 were measured (Promethionin, GA-4, Sable Systems Europe GmbH, Berlin, Germany), airflows were set to 60 L/min (Promethion FG-1000 and FG-250 flow generators, Sable Systems). A temperature of 22 ± 1°C and a relative humidity of 55% and an air pressure of about -60 Pa were maintained. Amount of dry food eaten was determined and faeces and urine were totally collected. In food and faeces carbon (C), nitrogen (N), crude nutrients and gross energy (GE) were determined and in urine only C, N and GE. N, C and energy balances and from these protein- and fat accretion were calculated. Data are given as median, minimum and maximum or as mean and standard error. Linear mixed models were calculated to identify dependencies of energy expenditure, protein- and fat deposition on the BCS at 8 mo of age with the software R version 3.3.1. Linear regression analyses were used to calculate energy requirements at given ideal fat deposition in the cats lean at 8 mo of age.

Results: At the age of eight mo17 of the kittens had a BCS of < 6 = lean (ten female: mean=5.1, SE=0.1, Min=4.8, Max=5.5 and ten male: mean=5.5, SE=0.1, Min=5, Max=5.8). The BCS of 10 kittens was ≥ 6 (five female:mean=6.1 , SE=0.1, Min=6.0, Max=6.4 and seven male:mean=6.4, SE=0.1, Min=6.0, Max=6.7). The body weight of female kittens at 4 and 6 mo, averaged for 2.2±0.08 kg and 2.9±0.09 kg, respectively, whereas males weighed 2.5±0.1kg and 3.8±0.12 kg at the same age. In female cats the weight gain accounted for 16.9±1.9 g/d at 4 and 6.6g/d at 6 mo, whereas male kittens gained at 4 and 6 mo 20±2.1 and 20±2.9 g/d. Intake of metabolisable energy, retained energy is given in Table 1. Metabolisability of energy was 0.8±0.01 at 4 and 0.79±0.01 at 6 mo of age.

Table1: Results of intake in metabolisable energy (EI), retained energy (RE), fat deposition (FD) and protein deposition (PD) in kitten at 4 and 6 month of age

	per kg FFM/d			per kg0.67/d		
	median	min	max	median	min	max
EI 4month (kJ)	706	570	976	780	684	1198
EI 6month (kJ)	507	419	737	674	514	1035
RE 4month (kJ)	255	154	617	282	179	777
RE 6month (kJ)	124	4	415	159	5	583
FD 4month (g)	4.1	1.8	10.9	4.4	1.9	13.6
FD 6month (g)	2.4	-0.9	7.2	2.3	-1.1	10.0
PD 4month (g)	4.0	3.1	9.9	4.3	3.5	10.6
PD 6month (g)	3.3	0.7	6.7	3.1	0.9	9.4

If the mean fat deposition of 5.5g/kg FFM at 4 mo is taken as ideal, the corresponding energy requirement for the cats lean at 8 mo was 715 kJ/kg FFM/d (884 kJ/kg BW0.67). Accordingly at 6 mo the ideal fat deposition was 1.9 g/kg FFM/d and the corresponding energy requirement could be calculated to be 503 kJ/kg FFM (720 kJ/kg BW0.67).

Conclusion: The data on energy and protein requirements of growing cats measured with help of indirect calorimetry complete the existing data seeming to be a more exact data base. Compared to the formula given by NRC (2006) for the female kittens at an age of 4 mo the energy recommendation would be similar with 1153 kJ ME per day after NRC and 1499 kJ ME per day if calculated with the new data of this study.

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7.

Effects of lactational stage and feeding level on adrenal cortex reactivity in dairy cows

Einfluss des Laktationsstadiums und des Fütterungsniveaus auf Nebennierenrinden-Reaktivität

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Changes in adrenocorticotropic hormone (ACTH) challenge test characteristics (i.e. total capacity of cortisol release, peak height and time of peak, etc.) in terms of repeatability in dairy cows changing their endocrine and metabolic status at different lactational stages were not investigated so far. The objective of the present study was to investigate relationships between ACTH test response characteristics and metabolic parameters at different lactational stages in dairy cows.

Methods: In 23 multiparous Holstein cows (12 cows fed a sole fresh herbage diet without concentrate, 13 cows fed with additional concentrate) (1) three ACTH challenge tests were performed: once during pregnancy in mid- to late lactation (T1) shortly prior to drying off, and in wk 3 (T2) and 8 (T3) after parturition. During the ACTH test, blood was sampled every 15 min from 0 to 180 min, and AUC was calculated. AUC of cows were sorted by size and their ranking compared among tests. Test characteristics were correlated to performance and metabolic parameters: dry matter intake (DMI), body weight (BW), energy balance (EB), plasma concentrations of free fatty acids (NEFA) and beta-hydroxybutyrate (BHB). Plasma cortisol concentration was measured by RIA (2). Data were analyzed using MIXED models in SAS (Version 9.4, SAS Institute, Cary, NC, USA) including test number and feeding level as fixed effects and the individual cow as repeated subject. Differences among tests and between feeding levels over time were detected by the Bonferroni t-test at a level of $P < 0.05$.

Results: Basal plasma cortisol concentrations were higher in mid to late lactation before dry off (T1) compared with T2 and T3 in wk 3 and 8 of lactation ($P < 0.05$). The adrenal cortex sensitivity (expressed as total AUC (AUCt) of cortisol response after ACTH application) was lowest at T2 compared with T1 and T3 ($P < 0.05$). Ranking of AUCt was not repeatable at any time-points of the ACTH tests. Neither cortisol peak values nor the difference between peak and basal values differed between the ACTH tests performed at T1, T2, and T3 ($P > 0.05$). Enhancing the energy deficiency during early lactation in wk 3 and 8 p.p. by omission of concentrate did not affect baseline cortisol concentrations in plasma, but decreased peak height at T2 ($P < 0.05$). The AUCt was lower by trend in cows without concentrate supplementation in wk 8 p.p. compared to supplemented animals ($P = 0.07$). Baseline cortisol concentrations in plasma were higher in cows with higher cortisol peak values after ACTH application, with a higher previous lactation performance, higher milk yield, and higher body weight ($P < 0.05$). Energy balance and the extent of BW loss since parturition had no effect on cortisol baseline concentrations. The AUCt was higher in cows with a higher baseline cortisol concentration, a more positive EB, and a higher DMI. Cortisol release after ACTH was lower in animals with high plasma concentrations of NEFA, BHB, and with higher contents of fat and free fatty acids in milk ($P < 0.05$). The cortisol peak height after ACTH administration was concomitantly higher in cows with a more positive EB, higher DMI, and lower plasma concentrations of NEFA and BHB.

Conclusions: The adrenal cortex reactivity was shown to be lower in dairy cows experiencing a higher metabolic load during the negative energy balance in early lactation as well as during aggravated imbalances in energy supply by omission of concentrate. Responses of the HPA-axis to ACTH challenge tests at different lactational stages were not repeatable, i.e. the differences of the endocrine and metabolic adaptation had a greater influence on the adrenal cortex responsiveness than the inherent individual genetic disposition. Hence, an a-priori characterization of the HPA-axis does not allow to predicting metabolic adaptation to metabolic stress in early lactation.

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8.

Association between the telomere length and mitochondrial DNA copy number during early and late lactation in dairy cows

Zusammenhang zwischen der Telomerlänge und der Anzahl mitochondrialer DNA-Kopien bei Milchkühen in der Früh- und Spätlaktation

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In the course of pregnancy and lactation, environmental, physiological, and energetic conditions change in high-yielding dairy cows. Increased metabolic demands influence cellular energy requirements and lead to elevated oxidative stress. Stress-related conditions have been related with both, telomere length, which is considered as a biological marker for aging, and mitochondria, the main site of energy metabolism in mammalian cells. Recently, we have demonstrated that both, the abundance of mitochondria (1) and the telomere length (2) change in dairy cows throughout lactation. Herein we hypothesized, that cellular aging processes represented by the telomere length, are influenced by cellular energy requirements, i.e. mitochondria per cell, during lactation in dairy cows. Therefore, we investigated the association between the abundance of mitochondria, reflected by the number of mitochondrial (mt) DNA copies per cell and the telomere length given by the relative quantity of telomeres (qT) in blood and key organs of energy metabolism, i.e. subcutaneous adipose tissue (scAT) and liver, from early and late lactating dairy cows.

Methods: Twenty-one lactating German Holstein cows (age: 2 to 6 years, lactation number: 1 to 5) were kept in a freestall barn and were fed according to their requirements. The animals were fed a partial mixed ration (6.3 - 6.8 MJ NEL/kg DM) for ad libitum consumption and concentrate feed (7.7 MJ NEL/kg DM) depending on the individual's milk yield. The cows had a mean body condition score of 3.0 ± 0.1 (5-point scale (3)). Blood samples from the jugular vein as well as liver, and scAT from the tailhead region were sampled during early lactation (3 - 4 weeks post partum) and late lactation (35 - 36 weeks post partum) in which the estimated total energy requirements per day were 132 ± 8.3 MJ NEL and 103 ± 5.7 MJ NEL, respectively. Biopsies were immediately snap frozen after sampling. Genomic DNA from whole heparinized blood and from the biopsies was extracted using commercially available kits. The number of mtDNA copies/cell was quantified by multiplex qPCR, targeting the 12S rRNA gene and using b-globin as reference gene (1). Telomere length was assessed by analyzing the relative qT products compared with the reference gene b-globin, using a multiplex qPCR (2). The associations between variables were assessed by Spearman correlations (SPSS 24).

Results: Irrespective of the time period, the number of mtDNA copies/cell and qT were strongly related in blood ($r = 0.675$; $P \leq 0.001$) and liver ($r = 0.665$; $P \leq 0.001$), the association in scAT was moderate ($r = 0.401$; $P = 0.005$). Regarding early lactation solely, the number of mtDNA copies/cell and qT were strongly associated in blood ($r = 0.735$; $P \leq 0.001$) and liver ($r = 0.643$; $P = 0.001$), whereas a moderate relationship was observed in scAT ($r = 0.416$; $P = 0.043$). In addition, moderate associations were detected in blood ($r = 0.554$; $P = 0.005$) and scAT ($r = 0.428$; $P = 0.037$) during late lactation, whereas mtDNA copies/cell and qT in liver from late lactating cows were strongly related ($r = 0.761$; $P \leq 0.001$).

Conclusions: Stress-related conditions in high-yielding dairy cows, are accompanied with changes in mitochondrial numbers and telomere length in blood and key organs of energy metabolism (1, 2). The number of mtDNA copies/cell was associated with the relative qT in blood, liver, and scAT from early and late lactating dairy cows. Thus, although precise mechanisms are not known so far, cellular energy requirements go along with cellular aging processes in dairy cows.

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9.

Impact of different roughage qualities at comparable concentrate level on feed efficiency and methane emissions of dairy cows during the lactation cycle

Einfluss unterschiedlicher Grobfutterqualitäten bei gleichem Kraftfutterniveau auf die Futtereffizienz und die Methanemission im Laktationsverlauf bei Milchkühen

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Feed costs are the major component of the dairy profitability. Therefore, efficiency in milk production may be increased by improving feed efficiency. Estimates of feed efficiency in dairy cows must account for different feed components of the ration and the mobilization and re-accretion of body tissues over the entire lactation cycle. As concentrate feed is much more expensive than roughage feed and may also be used to produce human food, increasing the energy density of roughage feed while maintaining the level of concentrate may improve feed efficiency without further use of food resources. On the other hand, roughage feed has a higher methane production potential and leaves a greater carbon footprint per unit of milk produced. The objective of the present study was to compare the impact of two rations with different roughage quality but the same level of concentrate on estimates of feed efficiency and methane production in dairy cows.

Methods: Twenty one German Holstein cows with comparable milk yield were dried-off after 1st lactation. Three weeks prior to expected parturition, animals were fed a close-up diet with 6.5 MJ NEL/kg dry matter (DM). After calving, 10 animals were randomly assigned to a lactation ration containing 6.1 MJ NEL/kg DM in roughage feed, and 11 animals to a ration containing 6.5 MJ NEL/kg DM in roughage feed. Different energy densities were achieved by using different grass silage qualities and by adding straw to dilute the energy-rich diet. Concentrate (250 g/kg energy corrected milk (ECM)) was added to both rations in equal amounts and fed as total mixed ration (TMR) until lactation week 42. Feed intake was measured daily while body weight (BW), body condition score (BCS) and back-fat thickness (BFT) were determined every two weeks. During lactation, milk yield was measured daily, whereas milk constitutes were analyzed weekly. In week -3, +3, +14 and +42 relative to parturition, animals were transferred into respiration chambers to measure CH4 production, feed intake and ECM over a period of 48 h. TMR samples were taken to analyze chemical composition. Based on the results and feed intake, net energy intake (NEI) was calculated. Data was analyzed using the MIXED procedure of the SAS/STAT software by repeated measurement ANOVA where the models contained the fixed factors group (6.1 and 6.5), time (-3, +3, +14 and +42 relative to parturition) and the interaction group × time.

Results: ECM yield and DMI were higher in the 6.5 compared to the 6.1 group ($P \leq 0.05$). BW, BCS and BFT did not differ between groups, however, BW and BFT showed a group × time effect ($P < 0.01$ and $P < 0.05$, respectively). CH4, CH4/DMI and CH4/ECM were not different between feeding groups. Feed conversion ratio (FCR = DMI/ECM), feed efficiency (FE = ECM/DMI), energy conversion efficiency (ECE = ECM/NEI), and energy conversion ratio (ECR = NEI/ECM) were not different between feeding groups, but metabolic efficiency (MEff = (NEI-ECM)/BW0.75) was greater in 6.5 than 6.1 animals ($P < 0.01$).

Conclusion: Although feeding roughage with higher energy density did not result in different feed or energy conversion rates or efficiencies, respectively, it resulted in a higher metabolic efficiency and ECM yield without increasing methane production. Based on these results we conclude that increasing the energy density of roughage in a ration improves profitability and metabolic efficiency, and allows minimizing the concentrate level in a ration without compromising environmental aspects related to methane.

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10.

Effect of energy concentration in roughage and allowance of concentrates on performance and energy metabolism of pluriparous dairy cows during early lactation

Einfluss von Energiegehalt im Grundfutter sowie Menge an Kraftfutter auf die Leistung sowie den Energiestoffwechsel bei mehrkalbigen Milchkühen während der Frühlaktation

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Introduction: In the beginning of lactation high yielding dairy cows have to overcome a period of negative energy balance due to reduced feed intake and high energy demands for milk production. Therefore it is necessary to feed rations containing high energy contents to counteract this energy deficiency. The aim of this study was to examine influences of different energy supplies from concentrates and roughages on performance and energy metabolism of pluriparous dairy cows during early lactation.

Methods: In the current experiment 64 pluriparous German Holstein dairy cows were used from three weeks antepartum until sixteen weeks postpartum. During dry period all cows received a diet with 80% roughage and 20% concentrates. The experiment was conducted as a 2x2 factorial design with two different energy concentrations in roughage (Rou) and two different amounts of concentrates (Conc). The experimental ration was fed *ad libitum* as a partial mixed ration (PMR) with a proportion of 60% maize silage and 40% grass silage on dry matter (DM) basis. Different amounts of straw were added to the PMR to receive either an energy content of 6.1 MJ NEL or 6.5 MJ NEL per kg DM in the roughage part of the rations. Concentrates were fed by an automatic feeding system depending on the expected milk yield of each cow to provide 150 g/kg ECM or 250g/kg ECM on DM basis. Feed intake was recorded daily for each animal. The cows were milked twice daily and milk yield was recorded by automatic milk counters. Milk samples were taken twice a week to analyze the ingredients of the milk. Blood samples were taken on day -50, -14, +8, +28 and +100 relative to calving from a vena jugularis and were analyzed for β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Data were analyzed by using the MIXED procedure of SAS 9.4 for repeated measurements with fixed effects of roughage, concentrates and time and the interactions between these factors. The cow within the treatment was considered as a random effect.

Results: During the first 28 days of lactation cows which received higher portions of concentrates showed an increase in total feed intake but lower roughage intake. Milk yield, milk ingredients and blood concentrations of NEFA and BHB were not affected by treatment (Tab. 1).

Conclusion: In the presented study feeding higher portions of concentrates leaded to an increase of total feed intake at expense of roughage intake within the first 28 days of lactation. In this period different amounts of concentrates or feeding roughage with a varying energy content did neither influence the performance of the cows nor the variables of energy metabolism.

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Table 1: Effect of energy content in roughage (Rou) and allowance of concentrates (Conc) on performance and energy metabolism within the first 28 days of lactation (LS-Means)

EL/kg DM	6,1		6,5			*p*-Value[2]			
er kg ECM	150	250	150	250	SEM[1]	Rou	Conc	Time	Rou*Conc*Time
Total feed	17,2	17,9	17,1	18,3	0,3	0,593	0,001	<0.001	0,770
Roughage	12,2	11,1	12,2	11,7	0,3	0,188	0,005	<0.001	0,786
Milk yield,	31,5	33,1	33,4	32,1	1,0	0,663	0,875	<0.001	0,151
Milk fat, %	5,10	4,99	4,80	4,81	0,16	0,137	0,749	<0.001	0,410
Milk prote	3,49	3,43	3,48	3,52	0,06	0,519	0,912	<0.001	0,007
BHB, mmc	1,07	0,88	0,86	0,98	0,11	0,596	0,730	0,006	0,304
NEFA, µm	618	534	657	653	78	0,314	0,569	<0.001	0,443

[1] SEM = Standard error of means; [2] p-values > 0.05 for all "concentrate*roughage", "roughage*week" and "concentrate*week" interactions

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11.

Effect of maize- or wheat-based diets on the abundance of selected proteins involved in insulin signaling of broiler chicken

Wirkung von Mais- und Weizen-basierten Diäten auf die Expression bestimmter Proteine von dem Insulin-Signalweg

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Alterable nutritional factors applied in poultry industry could have high impact on animal welfare and production. In the present study we investigated the effects of different cereal type (maize or wheat) -based diets on the insulin homeostasis of broiler chicken, which could be in direct or indirect association with growth performance, especially during the period of intensive growing. Soluble NSP (non starch polysaccharide) content - mainly arabinoxylans - is higher in wheat than in maize. These compounds can only be degraded in animals by microbial enzymes, providing substrates for probiotic microbial fermentation in the caecum resulting in higher total SCFA (short chain fatty acid), primarily butyrate production (1). Thus wheat-based diet (supplemented with xylanase and glucanase enzymes) could be considered as a significant endogenous butyrate source compared to maize-based diet. In earlier studies it was found that butyrate is able to influence certain members of insulin signaling pathway in a tissue specific manner in broiler chicken at the age of 3 weeks, however in another study much weaker effect was detected at the age of 6 weeks. Based on these experiences, in present study design we aimed to investigate the effect of maize and wheat-based diet on certain regulator proteins of the carbohydrate metabolism (key proteins of insulin signaling pathway and glucagon receptor) in different tissues of broiler chicken at different ages.

Methods: Male Ross 308 broiler (n=10/group at age 1, 3 or 6 weeks,60 in total) chickens were fed with maize-based or wheat-based diet (the latter with xylanase and glucanase supplementation). Feed intake of animals was measured by group. Ten animals per diet group were slaughtered on week 1, 3 and 6. Body weights of chickens were measured at all slaughterings. Tissue samples from liver and gastrocnemic muscle were taken at week 1, 3 and 6, and from abdominal adipose tissue on week 3 and 6 for Western blotting examinations. Expression of certain key insulin signaling proteins IRβ (Insulin Receptor β), PKB (Protein Kinase B), mTOR (mammalian Target of Rapamycin) and GR (Glucagon Receptor) was assessed by semiquantitativelyData were analyzed by two-way ANOVA and pairwise comparison using the R 2.14.0 software.

Results: The feed intake of diatery groups did not differ. Body weights of animals from maize-based and wheat-based diet did not differ at week 1 and week 6, but were higher in the wheat-based dietary group at week 3 (1.25 fold increase, $p<0.001$). In the liver increased IRβ and mTOR expression was measured in chickens fed with wheat-based diet compared to maize-based diet both at week 3 (IRβ: 2 fold increase, $p<0.001$; mTOR: 1.5 fold increase, $p<0.05$) and week 6 (IRβ: 1.5 fold increase, $p<0.05$; mTOR: 3 fold increase, $p<0.01$), however we found only a non significant increase in PKB expression at week 3 (2 fold increase, $p<0.1$). GR expression in the liver did not differ in wheat-based and maize-based groups, however we found a strong age effect: GR expression at week 1 was lower than at week 3 and week 6 4 fold increase, $p<0.05$). In gastrocnemic muscle, we did not found any diet effect on the studied protein expressions, but significant age effect was found in the case of IRB (7 fold decrease, $p<0.001$), PKB (5 fold decrease, $p<0.001$) and mTOR (2 fold decrease, $p<0.05$): protein expression was higher at week 1 than at the later ages. In abdominal adipose tissue diet type had only a non significant effect on IRβ at week 3 (2.5 fold decrease, $p<0.1$): in contrast to the liver, IRβ expression was lower in the wheat-based dietary group compared to animals kept on maize-based diet.

Conclusion: Based on the results obtained, present study highlights that different cereal types (maize or wheat) of diet, which finally results in different intensity of caecal SCFA, primarily butyrate production, is able to influence the expression of certain key insulin signaling proteins in broiler chicken in a tissue specific manner. The detectable effect of diet type appeared especially at week 3, during the intensive growing period of broiler chicken, when the metabolic pathways have also high intensity. These results highlights that nutritional factors have the greatest significance during this fast growing phase in broiler chicken. Our results also show that the expression and tissue distribution of certain regulator proteins of the carbohydrate metabolism have a remarkable age dependency.

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12.

Influence of dietary methionine source on the activity of methionine-resorptive mechanisms in the gastrointestinal tract of growing pigs

Einfluss der Methioninquelle im Futter auf die Aktivität Methionin-resorptiver Mechanismen im Verdauungstrakt von Läuferschweinen

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Methionine (Met) is a neutral, sulphur-containing, essential amino acid with several biological functions such as protein synthesis and synthesis of other sulphur-containing amino acids (cystine and cysteine; Cys) [1]. Dietary Met plus Cys supply has been increasingly optimized by supplementation of crystalline Met sources over the last decades for optimum health and performance [2]. The objective of this study was to determine if supplementation of different Met sources (DL-Met, L-Met and DL-hydroxymethylthiobutyric acid; HMTBA) influence the absorptive capacity of D-Met, L-Met and DL-HMTBA in different sections of the small intestine of pigs by modulating the activity of Met-absorptive mechanisms.

Methods: A total of 27 weaned piglets were randomly allocated to 3 treatment groups. A Met-deficient basal diet (0.45% Met+Cys) was supplemented with 0.21% DL-Met (diet 1), 0.21% L-Met (diet 2) or 0.31% DL-HMTBA (diet 3) to meet Met+Cys and crude protein (18%) requirements. After slaughter, absorption of D-Met, L-Met and DL-HMTBA was compared *ex vivo* in the duodenum, mid-jejunum and ileum of these pigs using Ussing chambers .Under short-circuit-conditions, the flux rates of Met or HMTBA from the mucosal to the serosal side were measured over 30 min using 14C-labelled D-Met, L-Met or DL-HMTBA in final concentrations of 50 µM or 5 mM. All flux rate data are given as means ± SEM with unit nmol/(cm²·h). Data were compared between regions and diets using ANOVA.

Results: With diet 1 at 50 µM concentration, flux of D-Met was highest in jejunum (1.71 ± 0.27 nmol/(cm²·h); $P < 0.01$) while ileal and duodenal flux rates amounted to 1.18 ± 0.15 and 0.54 ± 0.14, respectively. Flux rates of L-Met were lower in duodenum (0.48 ± 0.11; $P < 0.05$) compared to jejunum (2.98 ± 0.80) and ileum (5.09 ± 2.48). Finally flux rate of DL-HMTBA were similar in jejunum (1.40 ± 0.21) and ileum (1.38 ± 0.32) but lower in duodenum (0.37 ± 0.13; $P < 0.05$).With diet 2, flux rates of D-Met in duodenum (0.38 ± 0.09), jejunum (1.19 ± 0.39) and ileum (1.18 ± 0.41) and flux rates of DL-HMTBA (0.35 ± 0.07, 1.07 ± 0.31, 1.31 ± 0.30 in duodenum, jejunum and ileum, respectively) were not different at 50 µM to the flux rates with diet 1 ($P > 0.05$); however, the flux rate of L-Met was lower in the jejunum (0.68 ± 0.08) but not duodenum (0.35 ± 0.08) and ileum (1.95 ± 0.63) of pigs fed diet 2 compared to diet 1 ($P < 0.05$). Finally with diet 3, flux rate of D-Met in duodenum (0.29 ± 0.06) and ileum (0.88 ± 0.16) were not different to diet 1 or 2; however, D-Met flux rate in jejunum (0.61 ± 0.10) was smaller compared to diet 1 ($P < 0.05$) but not different from diet 2 ($P > 0.05$). Flux rates of L-Met in duodenum (0.43 ± 0.06) and jejunum (1.05 ± 0.22) were similar to the flux rates observed with diet 1 and 2 ($P > 0.05$), while the flux rates in ileum (1.12 ± 0.33) were significantly lower than the flux rate with diet 1 ($P < 0.05$). Flux rates of DL-HMTBA in the small intestine of pigs fed diet 3 were not different from those of pigs fed diet 1 and 2 ($P > 0.05$).

When a Met concentration of 5 mM was used, no differences in Met fluxes were observed between the three feeding groups, except for ileal DL-HMTA absorption, which was higher with diet 2 compared to 1 ($P < 0.05$). Across all diets, flux rates of D-Met amounted to 82 ± 13, 170 ± 25 and 170 ± 18 in duodenum, jejunum and ileum, respectively. Flux rates of L-Met amounted to 72 ± 10 in duodenum, 244 ± 25 in jejunum and 368 ± 57 in ileum; whereas, flux rates of DL-HMTBA amounted to 71 ± 10, 204 ± 25 and 225 ± 19 in duodenum, jejunum and ileum, respectively.

Conclusions: Absorptive capacity for Met and HMTBA is generally higher in jejunum and ileum compared to duodenum. Dietary Met source plays a crucial role in the regulation of Met absorption in all parts of the small intestine of pigs. In specific, dietary supplementation with DL-Met appears to increase absorptive capacity for D-Met and L-Met in several intestinal regions. Future analysis of the expression profiles of Met transporter genes and proteins shall provide insight into their regional distribution and regulation by DL-Met feed supplementation. Finally, transepithelial flux rates of the three Met sources rose over-proportionally when increasing mucosal concentration from 50 µM to 5 mM, indicating significant retention of all Met sources in the intestinal epithelium at low luminal concentrations.

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13.

Influence of dietary nitrogen and/or calcium on the expression of calcium transport-related proteins in the kidney of young goats

Einfluss einer diätetischen Stickstoff und/oder Calcium-Versorgung auf die Expression von Calcium-transportierenden Proteinen in der Niere bei wachsenden Ziegen

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A reduction in dietary nitrogen (N) caused massive changes in the calcium (Ca) homeostasis in young goats (1). During an N-reduced or a combined N- and Ca-reduced feeding, plasma Ca as well as serum calcitriol concentrations were reduced, while a Ca-reduced diet led to increased calcitriol levels and adjusted plasma Ca concentrations. Furthermore, it was shown that dietary N-reduction decreased the expression of intestinal Ca transport-related proteins based on decreased calcitriol concentrations (2). Therefore, it was hypothesised that the ruminant kidney might be able to maintain Ca homeostasis by modulating the expression of Ca transport-related proteins during an N-reduced diet. Therefore, the aim of the present study was to examine the effects of N- and/or Ca-reduced diets on the expression of renal transient receptor potential vanilloid channel type 5 (TRPV5), calcium-binding protein calbindin-D28K (CaBPD28K) and sodium-Ca exchanger (NCX1) in young goats.

Animals and Methods: Four groups of male coloured German goats received a control diet (N+/Ca+), a reduced N diet (N-/Ca+), a reduced Ca diet (N+/Ca-) or a reduced N and Ca diet (N-/Ca-). All diets were isoenergetic and were provided for 6-8 weeks. Ionised Ca concentrations were determined in whole blood samples using an ion-sensitive electrode. Serum calcitriol levels were measured with a commercial radioreceptor assay. Plasma insulin-like growth factor 1 (IGF1) concentrations were analysed in the Clinic for Cattle, Endocrinology Laboratory, University of Veterinary Medicine, Hannover, Germany. Renal cortex samples were taken shortly after slaughtering, frozen in liquid N2 and stored at -80°C. The expression levels of mRNA and protein of renal TRPV5, CaBPD28K and NCX1 were determined by *q*PCR and Western blot analysis. Data were analysed by two-way ANOVA with Tukey`s multiple comparison test.

Results: Ionised Ca concentrations were significantly decreased in goats kept on reduced N diets, and remained unchanged by dietary Ca-reduction. Plasma IGF1 was significantly diminished in goats fed the N-reduced diet. Serum calcitriol concentrations were about 32% decreased in the (N-/Ca+) animals while in (N+/Ca-) goats plasma calcitriol concentrations exceeded the levels in control (N+/Ca+) animals by 93%. For ionised Ca, IGF1 and calcitriol levels, no interactions between main effects were observed. The expression of TRPV5, CaBPD28K and NCX1 was significantly reduced in the N-reduced fed goats, whereas the CaBPD28k and NCX1 expression were increased due to the Ca-reduced feeding. No significant interactions between the two main effects were observed for the expression levels of Ca transport-related proteins.

Conclusions: The decrease of TRPV5, CaBPD28K and NCX1 expression during a dietary N-reduction could be based on reduced calcitriol concentrations. The reason for the diminished calcitriol levels could be the concomitantly reduction of plasma IGF1 levels, which are normally able to modulate calcitriol synthesis in the kidney. The simultaneous reduction of dietary N and Ca did not prevent the negative effect on renal calcitriol formation during an N-reduction. Therefore, the caprine kidney was not able to maintain Ca homeostasis in times of dietary N-reduction.

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14.

Recovery of the bovine epimural bacterial microbiome from either a continuous or a transient SARA challenge

Erholung des bovinen epimuralen bakteriellen Mikrobioms im Anschluss an eine kontinuierliche und eine unterbrochene SARA Challenge

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Question: The impact of a long-term subacute ruminal acidosis (SARA) on the bovine epimural bacterial microbiome (BEBM), and its consequences for rumen health are poorly understood. This study aimed to investigate shifts in the BEBM during recovery (2 weeks Recovery and 8 weeks Recovery) from a long-term continuous and transient SARA model.

Methods: Eight cows were fed forage and varying amounts of concentrates in this 2 x 2 crossover design experiment. A 60% concentrate ration was used to induce SARA following a 6 day adaptation period where the cows were adapted stepwise to the dietary induced SARA challenge. Both SARA challenge models lasted 4 weeks each, with the transient SARA challenge being interrupted by a 1-week challenge break in the second week of the challenge in a change-over design explained in details earlier [1]. After each SARA model, cows were fed forage diets only for 8 weeks to recover from SARA. Rumen papilla biopsies were taken before starting the SARA challenges (Baseline; forage feeding), at the end of the SARA challenges (SARA1 and SARA2), and after 2 (Recovery1) and 8 weeks (Recovery2) of the recovery period for both the continuous and transient models. In total, 56 rumen papilla biopsies were taken for DNA extraction, with all 8 cows experiencing both SARA models. Ruminal pH was continuously measured during the 4 week challenge using indwelling sensors. The BEBM was determined using Illumina MiSeq sequencing of the 16S rRNA gene (variable regions 3, 4, and 5). Sequence data were analyzed with the software package Mothur (http://www.mothur.org/). Statistics were analyzed with treatment and sequence of challenge model received as fixed effects. Multiple sampling times within the feeding model were used as repeated measures. Level of significance determined with Tukey was defined as $P \leq 0.05$ and $0.05 < P \leq 0.10$ was defined as trend.

Results: Daily mean ruminal pH was lower after the transient SARA challenge (5.93) than after the continuous SARA challenge (6.15; $P < 0.05$) [1]. In total 1,551,732 quality controlled sequences were obtained and clustered into 7,433 operational taxonomic units (OTUs). A Campylobacter-OTU and a *Kingella*-OTU were the most abundant OTUs (17.5 and 8.2% relative abundance). Out of 202 statistically analyzed OTUs (≥0.05% relative abundance), 91 OTUs showed a significant shift between the SARA and recovery with 46 OTUs significantly increasing and 45 OTUs significantly decreasing during the recovery of both SARA models. Furthermore, 34 OTUs showed a significant shift from the 2-week to the 8-week Recovery period. The relative abundance of 10 OTUs differed significantly between the continuous and the transient SARA models, with 6 OTUs increasing and 4 OTUs decreasing from the SARA challenge to the Recovery period.

Conclusions: Our results revealed strong shifts in the BEBM during the recovery of a SARA challenge and showed that these shifts mainly happened within the first 2 weeks of Recovery. After 8 weeks of Recovery, the BEBM of cows from both SARA models showed a full recovery as indicated by a combined clustering of the BEBM structure in samples from the Baseline and the Recovery periods. We conclude that the re-establishment of the BEBM after a SARA challenge, regardless of challenge model, occurs quickly and only minor shifts can be seen between the 2-week and the 8-week Recovery periods.

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15.

Temporal changes in bovine rumen epithelium gene expression in response to increasing concentrate feeding

Zeitliche Veränderungen der Genexpression im Rinderpanzenzottenepithels bei einer kraftfutterreichen Fütterung

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Question: The feeding of high grain diets to dairy cows commonly results in lowered pH and sub-acute ruminal acidosis. During longer periods of decreased pH, epithelial cell function in the rumen is altered and disrupted impacting nutrient absorption, increasing endotoxin and pathogen translocation, and increasing systemic inflammation. This study investigated the role of high grain diets on gene expression in the rumen epithelium with relation to nutrient transport, barrier function, and immune response.

Methods: To identify alterations in gene expression related to the adaptive metabolic capacities of the rumen epithelium, epithelial integrity, and the innate immune system of the cow, 8 non lactating Holstein cows were sampled for rumen epithelial RNA. All cows started at a baseline being fed a forage-only diet, followed by a 6-d transition to a 60% concentrate diet to lower pH. Rumen epithelium samples were taken via ruminal cannula on day 0 (Forage; n=8), and on either day 7 (Adaptation; n=4) or day 14 (High Grain; n=4) for targeted bovine gene expression analysis (qPCR). Samples were analyzed with the Proc Mixed procedure of SAS. The statistical model included fixed effects of treatment and animal.

Results: Reticulorumen pH parameters showed a significantly lower mean pH at during Adaptation (6.21; P=0.04) compared with the pH measured during the Forage feeding phase (6.49). The mean reticulorumen pH recovered by day 14 during the high grain feeding (6.33). Variation was noted in dry matter intake (DMI) between animals, as well as in contrast between day 7 and both day 0 (P = 0.05) and day 14 (P = 0.08). Of the 24 genes analyzed, 17 showed a quadratic expression pattern over the 14 days with the lowest expression being during the Adaptation. Genes associated with barrier function showed the greatest variance in expression over the 14 day experiment. Claudins 1, and 4 showed a dramatic depression in expression at day 7 with a recovery to similar or higher levels of expression at day 14 (P = 0.09 and P = 0.06, respectively). The only tight junction related gene which showed an opposite effect was CDSN, however due to large individual animal variation (P=0.04) no effect of treatment was seen corresponding to the drop in pH and DMI during Adaptation. This effect of animal variation was also seen in the expression of transport protein target 3-hydroxybutrate dehydrogenase type 2 (BDH2; P=0.04). Innate immune response related gene CD14 showed a significantly depressed expression during the Adaptation (P=0.05). Toll-like receptor 4 (TLR4) also showed a trend towards a decrease in expression at day 7 (P = 0.06). Significant correlations between the expression of DMI and CD14 (R=0.71, P=0.002), between the expression of DMI and TLR4 (R=0.60, P=0.01; respectively) and between CD14 and TLR4 expression levels (R=0.85, P

Conclusions: Depression of gene expression in response to the initial dietary adaptation seen between day 0 and day 7 shows an immediate cellular response of the animal to dietary adaptation. The variable gene expression found between the 7 day adaptation phase and the subsequent 7 day high grain diet clearly shows rumen modulation to reestablish homeostasis, maintain animal health, and adapt rumen epithelial function in response to dietary intake.

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16.

A study on NH_4^+-induced currents across the gastrointestinal epithelium of pigs

Eine Untersuchung über NH_4^+-induzierte Ströme über gastrointestinale Epithelien beim Schwein

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Introduction: Large quantities of dietary protein are degraded in the gastrointestinal tract of pigs to ammonia, absorbed, and hepatically converted to urea. Most of this urea is renally excreted into the environment and degraded to various nitrogenic compounds with detrimental impact on the health of the animals, their human caretakers, and ultimately, the global climate. Despite increasing reasons for concern, the knowledge about the molecular mechanisms leading to the absorption of ammonia from the gut of pigs is rudimentary. A recent study has provided evidence for the involvement of non-selective cation channels of the transient receptor potential family in the electrogenic absorption of NH4+ by the ovine and bovine ruminal epithelium[1]. Based on these findings, the aim of the present study was to gain first insights into the transport of NH4+ across various gastrointestinal epithelia of the pig.

Methods: Stomach (fundus), duodenum, proximal jejunum, ileum, caecum and distal colon of pigs were obtained from a local slaughterhouse, immediately stripped from the muscle layer and transported to the laboratory in ice-cold Ringer's solution (95% O2 / 5% CO2). To assess the impact of different concentrations of NH4+ on the short circuit current (Isc), the conductance (Gt) and the transepithelial potential (PD), tissues were incubated both in classical Ussing chambers (allowing a direct comparison of tissues in parallel) and in a vertical Ussing chamber (custom built to allow artefact free solution changes). A NaCl/NMDGCl Ringer solution was varied by replacing appropriate amounts of NMDG+ by 20 or 40 mmol·l-1 NH4+. In divalent-free buffers, Ca2+ and Mg2+ were replaced by 5 mmol·l-1 EDTA. On the mucosal side, pH was set to 6.4, with serosal pH at 7.4. Statistical evaluation was performed after testing for normality and ANOVA or ANOVA on ranks.

Results: In all six epithelia (fundus, duodenum, jejunum, ileum, caecum, colon), mucosal addition of NH4+ led to a significant and reversible increase in Isc both when added at a concentration of 20 mmol·l-1 ($p < 0.001$, N/n = 4/5, 6/7, 15/22, 6/9, 17/24, 17/24) and at 40 mmol·l-1 NH4+ ($p < 0.001$, N/n = 7/11, 6/9, 10/15, 6/10, 9/15, 11/15). Effects were concentration dependent ($p < 0.001$). In 40 mmol·l-1 NH4+ solution, basal PD was highest in the colon ($p < 0.001$, N/n = 4/6), while the lowest Isc and Gt values were found in the fundus ($p < 0.001$, N/n = 4/8). Duodenum (N/n=4/7), jejunum, ileum and caecum (all N/n = 4/8) ranked between these extremes. The mucosal removal of divalent cations from 40 mmol·l-1 NH4+solutions enhanced Isc, Gt and PD in all tissues studied ($p < 0.001$). Effects were fully reversible. Finally, the effect of a removal of divalent cations was studied in symmetrical NaCl solution without an electrochemical gradient using jejunal epithelium (N/n = 4/10), again resulting in a reversible, significant increase in Isc ($p < 0.001$)

Conclusion: The highest effect of adding NH4+ was observed in the colon, followed by the ileum. In conjunction with the good reversibility of all effects, the strong increases in PD and Isc after removal of divalent cations suggests the opening of a divalent sensitive, cation selective pathway rather than non-specific destruction of tissue integrity. The significant increase of Isc observed after removal of divalent cations in symmetrical NaCl solution without an electrochemical gradient present suggests that at least in part, this pathway must be energized by the Na+/K+-ATPase and is thus transcellular. We conclude that in analogy for what has been shown in the ruminal epithelium of cows and sheep, transport of NH4+ ions across the gastrointestinal tract of pigs might involve non-selective cation channels. More research is necessary to identify suitable candidate genes for these channels and to assess possible additional contributions of divalent sensitive junctional proteins localized in the paracellular pathway.

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17.

Is butyrate protective in porcine colon epithelium under hypoxia?

Wirkt Butyrat im porcinen Colon bei Hypoxie protektiv?

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Introduction: Many pathologic conditions in the intestine are linked to hypoxia, e.g. inflammation, cancer or strangulation ileus. In a previous study we could observe a protective role of butyrate in rumen epithelium under hypoxic conditions, which is also suggested by several studies describing butyrate as an anti-cancer agent and therapeutic in inflammatory bowel diseases. Butyrate is produced in a considerable amount not only in the forestomach, but also in the large intestine of nearly all herbi- or omnivore species. Thus, we wanted to elucidate its role in the susceptibility of colon epithelium to hypoxia using pigs as model animals.

Material & Methods: The colon of freshly slaughtered pigs (N = 4) was rinsed with ice-cold buffer solution and cut open longitudinally. The epithelium was stripped off the serosal and muscle layers. Then it was mounted in Ussing chambers (n = 24) under short-circuit conditions, gassed with 100% O2 and incubated either in a buffer solution containing 50 mM Na-butyrate or a basal buffer solution containing 50 mM NaCl instead. After an equilibration period of 45 minutes, part of the epithelia was submitted to "hypoxia" by changing the gassing to 1% O2 (in 99% N2). In one group of epithelia the gassing was changed back to 100% O2 after 15 minutes ("short-term hypoxia"), in another group it lasted on ("long-term hypoxia"). The electrophysiological parameters short circuit current (Isc) and tissue conductance (Gt) were monitored. At the end of the incubation theophylline was added to the buffer solution and the resulting increase in Isc was compared between the groups. For statistical analysis data were pooled for each animal (n = 4 for each N) and the treatment groups were compared using One Way Repeated Measurements ANOVA with subsequent Holm-Sidak comparison (Sigma Plot 11.0, Systat Software Inc., USA). The differences were assumed to be statistically significant if $p < 0.05$.

Results: Epithelia incubated with 50 mM Na-butyrate tended to have a lower initial Isc compared to those incubated in the basal buffer solution. Concomitant with this, the long-term simulation of hypoxia led to a more distinct decrease in Isc in the latter group, although it rapidly sunk and stabilized at the same level of approximately 0.5 µEq cm-2 h-1 under hypoxic conditions in both buffer solutions. This value was significantly lower than that of the control group (One Way Repeated Measurements ANOVA with subsequent Holm-Sidak comparison, $p < 0.001$). Tissue conductance was increased by long-term hypoxia. However, the increase in the group incubated with butyrate was significantly lower than that in the control group (One Way Repeated Measurements ANOVA with subsequent Holm-Sidak comparison, $p < 0.001$). The addition of theophylline to the buffer solution led to an increase in Isc in all groups except those undergoing long-term hypoxia. In these groups the increase of Isc after addition of theophylline to the buffer solution was nearly abolished irrespective of the presence or absence of butyrate.

Conclusion: While short-term hypoxia does not seem to have an effect on electrophysiological parameters, long-term hypoxia led to a decrease in Isc both with and without butyrate in the buffer solution, being mirrored in the loss of the epithelium's secretory capacity as shown by its reaction to theophylline. However, the hypoxia-mediated increase in Gt could be ameliorated by butyrate incubation. This points to protective effects of butyrate in the porcine colon epithelium similarly to those observed in ovine rumen epithelium. These effects and their mechanisms will be elucidated in future studies.

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18.

Modulation of cyclic adenosine monophosphate in sheep ruminal epithelium by short chain fatty acids and G-protein coupled receptor agonists

Veränderung der cAMP-Spiegel im ovinen Pansenepithel durch kurzkettige Fettsäuren und Agonisten G-Protein gekoppelter Rezeptoren

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To meet the demands of modern livestock husbandry, concentrated feed rather than crude fibre is mostly fed. Although this feed may improve ruminant's performance, it also leads to an increased concentration of short chain fatty acids (SCFAs) in the forestomach. One of the main nutritional disease syndromes in cattle is therefore ruminal acidosis. It is the question if ruminal epithelium is able to detect and react to large quantities of SCFAs and thus to avoid derailment of epithelial integrity in case of acidic ruminal conditions. Recent studies suggest G-protein coupled receptors (GPRs) to act as nutrient sensing targets which may also play a key role in maintaining homeostasis of ruminal epithelial cells. These receptors are assumed to downregulate the production of cyclic adenosine monophosphate (cAMP), a pivotal second messenger.
The aim of our study was to investigate the potential of SCFAs on lowering cAMP levels and to determine whether mucosal or serosal application leads to different effects. Additionally, we wanted to examine if a GPR agonist (niacin) is able to mimic the effects produced by SCFAs on cAMP level to determine if GPRs are involved.

Methods: Stripped ruminal epithelia obtained from ventral rumen sac of sheep were mounted in Ussing chambers and incubated in a buffer solution containing 0.5mM 3-isobutyl-1-methylxanthine to avoid cAMP degradation. Activation of adenylyl cyclase (to increase the production of cAMP) was triggered by 10µM forskolin. After forskolin addition, 10mM butyrate were added either on both sides or to the mucosal or serosal compartment, respectively. Niacin was added to both sides at concentrations of 0.5 and 1mM. After incubation, epithelia were minced in lysis buffer. Determination of cAMP levels in the supernatant was performed using AlphaScreen cAMP Assay Kit (PerkinElmer).

Results: Butyrate could decrease the level of cAMP after incubation with forskolin compared to solely forskolin-stimulated tissues (one-way repeated measures ANOVA + Tukey's multiple comparisons test, $N=6$, $p<0.05$). Administration of butyrate on mucosal side seemed to have more pronounced effects than serosal application (preliminary data, N=3). 1mM Niacin lead to cAMP levels similar to those of butyrate administration (preliminary data, N=3).

Conclusion: The obtained data substantiate the suspicion that butyrate is detected by G-protein coupled receptors in ruminal epithelium. It may change intracellular cAMP levels by activating above mentioned receptors. This may lead to an activity modification of ruminal transporters for SCFAs and protons such as monocarboxylate transporter 1 (MCT-1) or sodium-hydrogen exchanger 3 (NHE-3) leading to a better adaptation to the supplied nutrients.

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19.

Localization of apelin receptor in the hair follicles of ewes subjected to different nutritional levels

Lokalisierung von Apelin-Rezeptoren in den Haarfollikeln von Schafen in Abhängigkeit von der Energiedichte der Ration

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Question: Apelin (AP) is a novel bioactive peptide belonging to the family of adipokines and binding to a specific G protein-coupled receptor named APJ (1). AP was firstly isolated from bovine stomach extracts and it was later detected in several tissues and organs including adipose tissue that is a possible source of plasma apelin. Also APJ shows a widespread distribution in numerous areas of the central nervous system and in many peripheral tissues. Many physiological roles were described for AP, including the regulation of the cardiovascular system, appetite and drinking behavior, gastrointestinal and immune function. To our knowledge, there are no reports describing the expression or the role of AP and APJ in the skin that, at present, is considered an endocrine organ as it both produces and is a target for several hormones and substances with hormone-like activity. On the basis of these considerations, in this work, we investigated the expression of APJ in the skin of ewes subjected to different nutritional levels in order to point out the presence of structures that might be locally responsive to the action of AP and highlight differences between experimental groups.

Methods: Thirty days prior to the expected date of parturition, 16 pregnant Sarda ewes were randomly assigned to two experimental groups which were subjected to the following dietary treatments: a) low concentrate intake (LCI: 300 g/d); b) high concentrate intake (HCI: 800 g/d). Both groups received the same alfalfa hay ad libitum and had free access to water. Skin biopsies were collected from the lateral thoracic region of five ewes per group. Additional samples were collected from two non-pregnant non-lactating ewes that were used as controls. Samplings were performed 5 days before and 10, 30 and 40 days after parturition. Skin specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Skin sections of 5 µm thickness were used for immunohistochemical procedure. Sections were incubated with a rabbit polyclonal to APJ Receptor antibody (ab 140508, AbCam) for 24 hours and, then, with a goat anti-rabbit biotin conjugated antibody. The reaction was detected with the Vectastain ABC kit (Vector) and visualized with diaminobenzidine.

Results: Intense immunohistochemical staining for APJ was detected in the skin of the ewes. The reaction mainly involved the hair follicles while the epidermis appeared to be negative. In the hair follicles the staining was observed in the cytoplasm of cells belonging to the outer root sheath and extended throughout the hair follicle, from the infundibulum to the bulb. No differences were evidenced among sampling times in the HCI and the control ewes. Instead, a lower immunoreaction for APJ was observed after 30 and 40 days from parturition in samples obtained from the LCI ewes.

Conclusions: currently there are no studies that describe the expression of AP or its receptor in the skin of any animal species including humans. However, the clear identification of APJ in the hair follicles of the ewes suggests that apelin may be involved in the activity of this organ. In addition, the lower expression of APJ in the ewes fed with a low energy diet in late pregnancy and early lactation suggests that the nutritional level may affect the activity of hair follicles through the action of AP. The identification of APJ in ewe skin is a preliminary study but it represents an important contribution to introduce the study of AP in the skin of domestic animals. 1) Pitkin S.L., Maguire J.J., Bonner T.I., Davenpor A.P. (2010) Pharmacological Review 62:331-342,

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20.

A study of the bovine TRPV3 channel as a pathway for the uptake of Ca^{2+}

Eine Untersuchung des bovinen TRPV3 Kanals als Mechanismus für die Aufnahme von Ca^{2+}

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Introduction: Calcium is an irreplaceable nutrient for dairy cows, with considerable quantities absorbed by the rumen. Carefully done recent studies of Ca2+ transport across the ovine rumen clearly show that mucosal to serosal flux of Ca2+ is primarily transcellular (1) and involves both electroneutral and electrogenic mechanisms (2). However, epithelial calcium channels TRPV5 and TRPV6 are not expressed either by the ovine or the bovine rumen. Instead, a recent study of the native ruminal epithelium demonstrates the expression of mRNA for the bovine representative of TRPV3 (bTRPV3) (3), a non-selective cation channel from the transient receptor potential family. In addition, it could be shown that menthol, which is a known agonist of the human analogue of TRPV3, stimulated Ca2+ fluxes across the native ruminal epithelium *in vitro* (3). The current study tests the hypothesis of the bovine analogue bTRPV3 as a menthol-sensitive uptake pathway for Ca2+.

Methods: bTRPV3 was transiently overexpressed in HEK293 cells via an established expression system using the vector pcDNA5/TO. Control cells contained the empty construct (MT). The intracellular calcium concentration [Ca2+]i was measured using ratiometric imaging with the fluorescent dye Fura-2. Statistical evaluation of data was performed after testing for normality using the Shapiro-Wilk test and t- or Rank Sum Test, as appropriate.

Results: No significant differences were observed in resting [Ca2+]i in bTRPV3 cells and MT controls, suggesting efficient mechanisms for intracellular Ca2+ homeostasis. Menthol (1 mmol×l-1) induced a significantly higher ($p < 0.01$) and more rapid ($p < 0.05$) increase in Ca2+ influx in cells overexpressing bTRPV3 ($n = 11$) than in MT controls ($n = 10$). No effect was observed when the solvent for menthol (ethanol) was given at appropriate concentrations. At a concentration of 10 mmol×l-1, Mg2+ partially blocked the effect of menthol both when given before ($n = 10$, $p < 0.05$) or after the application of menthol ($p < 0.05$). Smaller effects could be observed at lower concentrations of Mg2+ (2 mM or 5 mM) that did not pass testing for significance ($n = 9$).

Conclusion: The bovine analogue of TRPV3 (bTRPV3) is a promising candidate for the mediation of Ca2+ absorption across the bovine rumen. A ruminal concentration of 10mM Mg2+ is rather unlikely. However, the interaction between Ca2+ and Mg2+ is interesting, since older studies have shown negative effects of high Ca2+ intake on ruminal Mg absorption or Mg2+ digestibility.

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21.

The gut barrier integrety and mucosal immune response after heat stress in the jejunum of dairy cows

Die Integrität der Darmbarriere und der mukosalen Immunantwort bei Hitzestress im Jejunum von Milchkühen

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Heat waves with temperatures above 30°C are predicted to occur more frequently during summer periods increasing the risk for health problems, e.g. endotoxemia and systemic inflammation, both possibly caused by a leaky gut. Heat stress can reduce blood flow in the body core potentially leading to a disruption of the tight junction connections and therefore the gut barrier integrity. Cell-to-cell contacts are formed by the interaction of tight junction proteins of adjacent enterocytes thereby protecting the host against paracellular bacterial infiltration and penetration of toxic substrates. However, it is not known whether disruption of the intestinal barrier and the subsequent immune response are specific to ambient heat or rather due to reduced feed intake during increased ambient temperatures. Therefore, the objective of this study was to compare the effect of short-term heat stress and pair-feeding at thermoneutrality on gut barrier and immune response in the jejunum of lactating dairy cows.

Methods: Ten German Holstein dairy cows in established 2nd lactation (245±102 days in milk) were grouped to heat-stressed (HS) and pair-feeding (PF). Animals were kept in a climate chamber at thermoneutral conditions (15°C; 63±1% relative humidity (RH) resulting in a temperature-humidity index (THI = 60) for 6d and received a total mixed ration twice daily (at 0700 h and 1500 h). Thereafter, five HS cows were exposed for four days to 28°C (with 52±2% RH resulting in a THI = 76) with *ad libitum* feeding and access to water, both tempered to 28°C. The reduction of daily *ad libitum* intake of HS cows was calculated as percentage to provide the same amount of feed energy to PF cows. The five PF cows were continuously exposed for four days to 15°C (THI= 60). After four days of HS or PF, cows were slaughtered to obtain jejunum samples. Tissue sections were stained with hematoxylin and eosin for villi height and crypt depth analysis. Jejunum mucosa scrapings were utilized for mRNA expression (ZO-1, ZO-2, Occludin, Claudin1, TNF, IL-6, IL-4, IL-10, relative to the reference genes HPRT1 and RPL0), Western blot analysis (Claudin 1, ZO-1) and IL-1β ELISA. Thirty villi heights and crypt depths from three different sections per cow were measured and the mean was calculated. Differences between PF and HS were analyzed using the Mann-Whitney-U test including the UNIVARIATE procedure of SAS (Version 9.4).

Results: Villi height was not significantly different between HS and PF cows (HS 497.4±43.4 vs PF 549.9±29.7 µm). Crypt depth was also comparable between groups (HS 242±27.5 vs PF 265.8±29.7 µm). The mRNA abundance of the tight junction protein ZO-1 was 2-fold higher in HS than PF cows (P=0.056). Claudin1 mRNA abundance tended to be higher for HS cows (1.5-fold, P=0.096), but remained unaltered for ZO-2 and Occludin. Western blot analysis showed comparable Claudin1 expression, whereas ZO-1 expression tended to be higher in HS cows (1.5-fold, P=0.096). Pro-inflammatory cytokine mRNA abundance of TNF and IL-6 were comparable between groups. Anti-inflammatory cytokine IL-4 tended to be higher in HS (2.4-fold, P=0.096), while IL-10 remained unchanged. IL-1β protein abundance was not different between groups (HS 7.6±1.6 vs PF 6.8±1.9 pg/µg protein).

Conclusion: Gut morphology was comparable between HS and PF cows after four days of challenge. However, HS animals seem to compensate for presumably challenged gut integrity by inducing higher Claudin1 and ZO1 mRNA expression, likely in an attempt to minimize bacterial translocation through the intestinal barrier. Future functional studies are needed to prove this hypothesis. Besides, higher anti-inflammatory cytokine mRNA abundance in HS cows suggests a mechanism to reduce heat-induced intestinal inflammation.

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22.

Effect of varying dietary supply with neutral detergent fibre, starch and sugar to fattening bulls on post-mortem endpoints of ruminal fermentation and glutathione metabolites within rumen papillae

Einfluss einer variierenden alimentären Versorgung von Mastbullen mit neutraler Detergentienfaser, Stärke und Zucker auf post-mortem Endpunkte der ruminalen Fermentation sowie Glutathionmetabolite in den Pansenzotten

*Brugger D., Ettle T., Windisch W., Bolduan C. – Freising/Grub

Decreasing the ruminal acetate:propionate ratio in fattening bulls by reducing the supply with neutral detergent fibre (peNDFom) increases volatile fatty acid (VFA) uptake from the rumen lumen (1). This might be accompanied by oxidative stress. The present study investigated post-mortem endpoints of rumen fermentation and glutathione metabolites within rumen papillae in fattening bulls fed varying amounts of neutral detergent fibre, starch and sugar.

Methods. A detailed description of the experimental design can be found in the abstract of Ettle et al. 2017 (2). A total number of 71 growing German Fleckvieh bulls were randomly assigned to three *ad libitum* feeding groups. Diets were designed to establish isoenergetic and isonitrogenic conditions and to differ in the concentration of peNDFom>1.18 mm (307, 235, 211 g/kg DM) as well as starch+sugar (366, 404, 438 g/kg DM) yielding structural values (SV) of 1.0, 0.52 and 0.48. This was achieved by varying ratios of maize silage:concentrates (DM: 68:32, 31:69 and 31:69 for SV 1.0, 0.52 and 0.48, respectively). In the SV 0.48 group sugar beet pulp in concentrates was reduced (36% to 22% of DM) and replaced with barley. This resulted in group specific differences in peNDFom and starch+sugar intake (NDFI, SSI) (Table). Bulls were slaughtered after 24h fasting. Rumen fluid was sampled for VFA and rumen papillae for glutathione analysis by HPLC (VWR-HITACHI LaChrome Elite System). Data was analysed by estimating Pearson correlation coefficients (r). There was no statistical comparison of group means.

Results. Ruminal acetate decreased (39.9 ±10.2, 38.4 ±14.5, 35.4 ±10.2 nmol/L) whereas propionate increased (10.4 ±4.1, 10.9 ±5.2, 10.8 ±3.3 nmol/L) numerically over groups (SV 1.0, 0.52, 0.48). Ruminal pH numerically decreased from group SV 1.0 to 0.52 (7.21 ±0.1, 7.16 ±0.2), whereas group SV 0.48 exhibited no difference to SV 1.0 (7.21 ±0.2). Reduced (GSH) and oxidised (GSSG) glutathione in rumen papillae numerically decreased over groups (542 ±186, 521 ±207, 515 ±194 nmol/g for GSH and 13.6 ±5.6, 13.1 ±6.7, 12.5 ±9.1 nmol/g for GSSG in SV 1.0, 0.52 and 0.48). The behaviour of the molar ratio GSH:GSSG was contrary by numerically increasing over groups (42.8 ±14.3, 44.1 ±19.4, 53.8 ±28.9). GSH in rumen papillae was not correlated to ruminal acetate or propionate (Table). In contrast, GSSG was positively and significantly correlated to acetate in group SV 0.52 and propionate in SV 0.52 and 0.48. The ratio of GSH:GSSG exhibited a stepwise increase in negative correlation to acetate and propionate over groups (SV 1.0, 0.52 and 0.48). These correlations were significant to acetate in case of SV 0.48 and propionate in case of SV 0.52 and 0.48.

SV		1.0	0.52	0.48
NDFI/SSI, kg/d		3.40/3.45	3.18/4.07	3.02/4.43
		N=23	N=22	N=24
GSH versus	Acetate	r=0.08, P=0.73	r=0.16, P=0.46	r=0.04, P=0.85
	Propionate	r=0.19, P=0.38	r=0.13, P=0.54	r=0.23, P=0.26
GSSG versus	Acetate	r=0.15, P=0.50	r=0.42, P=0.05	r=0.30, P=0.15
	Propionate	r=0.21, P=0.34	r=0.42, P=0.05	r=0.43, P=0.03
GSH:GSSG versus	Acetate	r=-0.12, P=0.59	r=-0.32, P=0.15	r=-0.41, P=0.05
	Propionate	r=-0.07, P=0.75	r=-0.36, P=0.10	r=-0.42, P=0.04

Conclusion. Ruminal VFA and pH were in line with earlier findings (1), highlighting an increased capacity for proton clearance from the ruminal lumen in groups SV 0.52 and 0.48). The increase of the GSH:GSSG molar ratio in these groups points towards an upregulation of antioxidative activity in order to maintain GSH reactivity. This may indicate an increase in the production of reactive oxygen species in rumen papillae of bulls with reduced NDFI and, at the same time, increased SSI.

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23.

Effects of four different diets on the relationship between methane emission and milk fatty acids profile in dairy cows: Deduction of a methane indicator

Effekte von vier verschiedenen Rationen auf die Methanemission und das Milchfettsäureprofil von Milchkühen: Ableitung eines Methanindikators

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Methane production and milk fatty acid (**FA**) profile of dairy cows are influenced by dietary composition. We investigated the relationship between individual methane output and milk FA profile using diverse feed ration compositions. The aim of the study was to deduce an improved milk FA indicator to predict individual methane production (**MP**).

Material and Methods: Twenty (106 ± 28 days in milk; 580 ± 57 kg BW), half-sib German Holstein cows were fed a standard TMR. In experimental wk 1, cows were transferred to one of 4 different isoenergetic diets: corn silage (**CS**), CS + linseed (**CSL**), grass silage (**GS**), GS + linseed (**GSL**), and continuously fed on this diet until wk 6. Thereafter, diets were changed from GS to GSL or vice versa and from CS to CSL or vice versa and fed between wk 7 and 12. Diets contained grass and corn silage at DM levels of 13% and 45% (CS and CSL), and 36% and 19% (GS and GSL), respectively. Linseed contributed 8% of DMI in GSL and CSL diets at the expense of concentrate. Each cow was tested rotationally with two diets (CS and CSL, or GS and GSL) in two consecutive time periods. The MP was measured in respiration chambers in wk 2, 4, 6, 8, 10 and 12 for 48 h each. During respiration measurements a pooled milk aliquot was collected and analysed for FA by infrared spectroscopy. Diet effects were analysed using PROC MIXED of SAS and the model contained the fixed effects of the basal diet (BD) (CS or GS), linseed (L) and the interaction of them. Regression equations for MP (L/d) were obtained using PROC REG of SAS with the STEPWISE variable selection method.

Results: Cows fed the CS as compared to the GS diet showed 16% less MP (CH4/ECM), whereas L reduced MP by 12% irrespective of diet (Table 1). Dry matter intake was higher for corn silage based diets. With both CS and GS diets, milk saturated FA (SFA) and C16:0 decreased with L supplementation by 14% and 24%, respectively. Both the portions of unsaturated FA (USFA) and C18:0 increased by 35% with L supplementation. Milk FA profile did not differ between GS and CS diets. Regression equations derived for each diet differed in the milk FA predicting MP. The goodness of fit for MP (L/d) prediction ranged from R2= 0.62 to 0.76 for individual diets. Pooled data analysis across all diets indicated R2= 0.61.

Table 1 Effects of 4 different diets on methane emission and milk fatty acids by ANOVA[1]

	Diets					P - value		
	CS	CSL	GS	GSL	SE	BD	L	BD x L
CH_4/ECM (L/kg)	19.6	17.5	22.7	19.7	0.86	0.008	0.002	0.526
CH_4/DMI (L/kg)	31.0	28.4	32.2	29.5	0.92	0.266	0.002	0.910
SFA (%)	71.0	61.5	71.2	60.3	0.82	0.557	0.001	0.329
USFA (%)	29.0	38.5	28.8	39.7	0.82	0.557	0.001	0.329
C16:0 (%)	31.3	24.0	31.7	23.9	0.68	0.792	0.001	0.699
C18:0 (%)	11.1^{c}	14.5^{ab}	10.6^{c}	14.8^{a}	0.22	0.518	0.001	0.065
n-3 FA (%)	0.7	0.9	0.7	0.9	0.02	0.233	0.001	0.827

[1] LSM values with different letters differ among diets at $P< 0.05$

Conclusion: Our data confirms that variation in MP and milk FA profile are diet dependent. Milk FA profile reflected the level of MP and data are suitable to construct a regression equation. Specific milk FA predicting MP differ according to the diet fed. Nevertheless, across all diets roughly 60% of the variation in MP can be explained by milk FA profile.

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24.

Methane emissions from grazing dairy cows: comparison of data using the sulphur hexafluoride tracer technique and the GreenFeed system

Methanemission weidender Milchkühe: Vergleich von Daten gemessen mit der Schwefelhexafluorid-Tracertechnik und dem GreenFeed System

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Enteric methane (CH4) emissions are produced as a result of ruminal fermentation and represent an energy loss to the animal, which can vary between 2-12% of gross energy intake. In order to quantify methane emissions from grazing ruminants and to develop nutritional strategies to mitigate CH4 emissions on pasture it is necessary to evaluate methods for efficient collection of data from animals in their accustomed environment regarding their accuracy and feasibility. The sulphur hexafluoride (SF6) tracer technique is an established method to measure ruminal CH4 emissions from free-ranging ruminants (1) but it is labour intensive. The GreenFeed (GF) system (C-Lock Inc., Rapid City, SD, USA) may be an alternative (2). It is a mobile device measuring respiration gas output during voluntary visits of a feeding station. However, up to now it is still unclear how many days of measurement are necessary to obtain reliable data on CH4 emission per cow per day. The objective of the present study was to determine the extent to which data obtained from dairy cows with the GF system reflect those using the SF6 technique by assessing relationships and differences of emission data from the two methods.

Methods: The study was carried out with 13 multiparous Holstein-Friesian dairy cows averaging 21.7 ± 4.7 kg/d of milk and 589 ± 25 kg of body weight. The cows grazed as a single herd on a pasture without concentrate supplementation. Before the measurements started, the cows got accustomed to the GF system and were equipped with a calibrated permeation tube releasing SF6, which was administered orally in the forestomach using a balling gun. During a 5-d period, when the SF6 tracer technique and the GF system were applied simultaneously, daily individual respiration gas samples were collected into evacuated canisters fixed on the cows' 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 CH4 emission was calculated from the SF6 release rate (4.31 ± 0.72 mg/d) and CH4/SF6 ratio of the gas sample, taking background concentrations of the two gases into account. The daily CH4 estimations from GF were based on integrating measurements of ventilation air flow and gas concentration during visits to GF, which were delimited by detection of head proximity. Visits were encouraged by offering small portions (33 g) of bait feed (pelleted dried whole maize plant); this up to eight such portions per visit. Overall, the GF measurements were performed during a period of 11 consecutive days. Within this period daily CH4 emission data were averaged per cow over 5 d (P1), 7 d (P2) and 11 d (P3) and compared with daily CH4 emission data determined with the SF6 technique and averaged per cow over the 5-d sampling period. Data were analysed with the ANOVA procedure of SYSTAT 13 and Spearman rank correlation coefficients were calculated.

Results: The average number of GF visits was 1.64 ± 0.72, 2.23 ± 0.56, 2.24 ± 0.52 times per day for P1, P2 and P3, respectively. Using P1, the estimated CH4 emission (g/d) from the GF (331 ± 54) was higher (P4 emission determined by the SF6technique (245 ± 39). The same was observed using P2 and P3 with greater ($P<0.001$) emission values found with GF (318 ± 35 and 311 ± 35 g/d). The Spearman correlation coefficient between the methods was moderately high in P1 (0.57, $P = 0.042$) but slightly improved when increasing the observation time with GF in P2 (0.59, $P = 0.036$) and P3 (0.62, $P = 0.025$).

Conclusion: Overall, the CH4 emissions estimated by GF were higher than those obtained using the SF6 technique. Number and temporal distribution of GF spot measurements relative to patterns of CH4 emission may partly explain this. Spearman correlation of ranked individual emissions shows moderate relationship between the methods. The correlation got slightly stronger and the variation within the GF measurements lower when the measurement period with the GF was extended from 5 to 11 d. Further studies have to show whether there is a systematic overestimation of CH4 emission with GF on pasture which can be corrected by standard adjustments.

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25.

Methodological study on the use of a portable Laser Methane Detector to assess methane concentration in air exhaled by Boer goats

Methodische Studie zur Nutzung eines tragbaren Lasermethandetektors für die Messung der Methankonzentration in der Atemluft von Burenziegen

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Several methods have been used to determine methane (CH4) emissions in ruminants (1). Comparison with respiration chamber measurements proved the portable Laser Methane mini-G (SA3C50A) Detector (LMm-G) technique (Crowcon Detection Instruments Ltd.) to be a reliable method to estimate CH4 concentration in air exhaled by dairy cows, steers and sheep (2,3), but no measurements were taken in goats so far. Also, no standardized measurement protocol is available yet and information on sources of variation in CH4 concentration is scarce. The current trial applied the LMm-G for CH4 measurements in goats under (experimental) farm conditions, in order to evaluate the effect of the position of goats, namely standing or lying. We assumed that the CH4 concentration in air exhaled by a goat lying down voluntarily (and often ruminating) significantly differs from when the animal is standing (but possibly also ruminating). Feed quality and feed intake were of subordinate relevance in this test.

Methods: The study was carried out in 12/2015 at the facilities of the Department of Animal Sciences, University of Göttingen. Four female Boer goats of 32.7 ± 2.75 kg body weight were housed indoors in individual pens (2 x 2 m²) at a temperature of 18 °C. Each goat received 3 kg FM of hay and 0.5 kg FM of concentrated feed mix (pellets, oat grains and sugar beet pulp in equal parts) daily. The latter was offered at 8:00 h and hay at 8:00 h, 13:00 h and 18:00 h (each time 1/3 of the total amount). Whereas concentrate was completely consumed, hay refusals approximated 1/3 of daily offer. Water and mineral blocks were offered *ad libitum*. The methodological test lasted four days with measurements taken at 15:00 h (lying; this was the position all but one goat voluntarily expressed at 15:00 h) and 16:00 h (standing, prompted by the team). The LMm-G operator assured a fixed distance of 1 m between the goats' nostrils and the LMm-G. Three repeated measurements, each lasting two minutes, were taken per goat and position. Data were analyzed by Kruskal-Wallis ANOVA (goats) and Mann-Whitney-U test (position). Pairwise comparison among goats was done by Wilcoxon rank sum test. The level of significance was set at $P < 0.05$.

Results: The mean exhaled CH4 concentration for lying was significantly higher than that for standing. However, when comparing the mean CH4 concentration measured in individual goats, we only observed significant differences in goat 2. Across goats, goat 1 had the highest total mean CH4 concentration and the highest mean CH4 output when standing. For lying, the mean CH4 output did not differ across goats.

Table 1: Methane (median, ppm * m) in air exhaled by lying and standing Boer goats across four days

	Standing	Lying [1]	Total	P-value (position) [3]
Goat 1	16.3[a]	15.5	16.3[a]	> 0.05
Goat 2	6.4[b]	15.8	9.5[b]	< 0.01
Goat 3	9.3[b]	7.7	8.7[b]	> 0.05
Goat 4	8.7[b]	-	-	-
Total	9.7	14.1	10.5	< 0.001
P-value (goats) [2]	< 0.001	> 0.05	< 0.001	

1 No data available for goat 4 for position lying. ² Kruskal-Wallis ANOVA and Wilcoxon rank sum test for pairwise comparison across goats. ³ Mann-Whitney-U test for comparison of positions lying and standing.

Conclusions: The position of goats does affect the mean CH4 concentration in exhaled air as measured close to the animal's nose by the LMm-G. Since the present results are not consistent across animals, it is important to account for both, the individual goat and its position, when setting up an experiment in which the LMm-G is to be used.

Acknowledgements: This trial was a methodological pre-test for the projects UrbanFoodPlus (BMBF, GlobE 031A242-A) and FOR2432/A03 (DFG). We thank Christian Wagner and Julian Plagemann (Georg-August-Universität Göttingen) for their technical assistance.

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26.

Effect of zinc oxide sources and dosages on intestinal bacterial counts and gut integrity of weaned piglets

Wirkung von unterschiedlichen Zinkoxidquellen und -dosen auf die Anzahl von Darmbakterien und Darmgesundheit von abgesetzten Ferkeln

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Zinc (Zn) is an essential trace element for monogastrics. As the native Zn content in vegetal material is generally low, Zn is added at nutritional dosage in piglets' diets. It can also be supplied in form of Zn oxide (ZnO) at pharmacological dosage (2400 mg/kg of Zn) in feeds of weaned piglets in order to improve performance through adjusting gut health. As a result, Zn concentration in animal waste can be high and leads to environmental concerns. Consequently, new solutions are investigated. A potentatied ZnO source, manufactured under a patented process that results in enhanced physico-chemical properties (including a high specific surface area), is evaluated in this trial.

Methods: In this study, the effects of regular ZnO were compared to a potentiated ZnO source (HiZox®) at low dose. High iron level was used to induce gastro-intestinal disturbances: (T1) 110 mg/kg of Zn from standard ZnO + 100 mg/kg of Fe from $FeSO_4$, (T2) 2400 mg/kg of Zn from standard ZnO + 100 mg/kg of Fe from $FeSO_4$, (T3) 110 mg/kg of Zn from standard ZnO + 500 mg/kg of Fe from $FeSO_4$, (T4) 2400 mg/kg of Zn from standard ZnO + 500 mg/kg of Fe from $FeSO_4$, (T5) 110 mg/kg of Zn from potentiated ZnO + 500 mg/kg of Fe from $FeSO_4$ and (T6) 220 mg/kg of Zn from potentiated ZnO + 500 mg/kg of Fe from $FeSO_4$. Each of these 6 treatments was replicated in 4 pens (2 piglets per pen, 20 days of age at start) during 15 days. Animal performance, Enterobacteriaceae counts by qPCR and coliforms and *E. coli* counts in intestinal contents using plate counting on selective media were measured. Gut barrier and chloride secretion upon secretagogues in distal jejunum were assessed, in Ussing chambers.

Results: Groups fed regular ZnO at 2400 mg/kg of Zn and potentiated ZnO at 220 mg/kg showed higher growth than other groups, irrespective of Fe content (PE. coli in distal small intestine than groups with 110 mg/kg of Zn from regular ZnO (PE. coli. The reduction of bacterial counts was confirmed by results from qPCR analysis. Transepithelial electrical resistance (TEER) of jejunal mucosa was significantly (P

Conclusion: The potentiated ZnO at low dosage showed positive effects on the reduction of bacterial counts and improved gut epithelial barrier integrity, albeit similar to the effects of pharmacological dosage of regular ZnO.

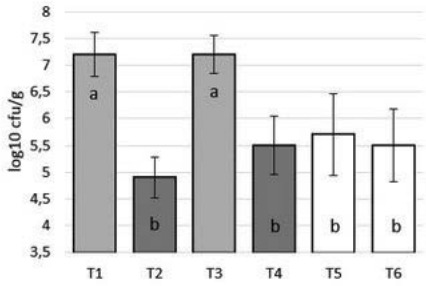

Fig. 1 - Coliform counts in distal small intestine

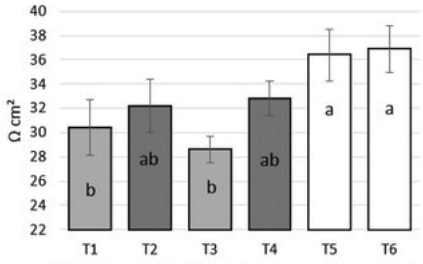

Fig. 2 - Transepithelial electrical resistance (TEER) of jejunal mucosa

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27.

Parakeratosis in piglets caused by a nutritive secondary zinc deficiency

Parakeratose bei Ferkeln ausgelöst durch einen nutritiv bedingten sekundären Zinkmangel

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Zinc is in the mammal's body, following iron, the second most abundant micro element and a cofactor for over 300 different enzymes, fulfilling a multitude of biochemical and biological roles (1). Its absorption takes place in the jejunum and is ensured by energy-dependent transport systems, which are Zn specific or general metal-transporters (2). Zn deficiency in piglets is well known. The most present symptom is the appearance of parakeratosis as circumscribed reddened plaques and thick scabs located at the ears, perineum, abdomen and medial thighs (3). In the present case the parakeratosis was a secondary finding within a study on different Ca and P supplementations of weaned piglets.

Methods: 21 weaned piglets at the age of 28 days (BW: 8.44 ± 1.31 kg) were housed and fed individually over a period of 35 days. Three botanically identical diets were offered as dry mash to seven piglets, each. The first diet contained a low (L) P-level (25% below the recommendation of the GfE), the second one a medium (M) P-level (according to the GfE), the third one contained a high (H) P-level (plus 25%). For a constant Ca : P ratio, the Ca content in the feed was equalised. Crude nutrients and mineral contents were analysed according to the official methods of VDLUFA. Serum minerals (Fe, Zn, Cu) were determined as well (ICP-OES). Skin samples of affected piglets were histologically examined. Data were analysed using one-way ANOVA (SPSS version 20).

Results: The mineral contents in the diets, in serum and the zootechnical data are shown in the table.

	content in the diet (in DM)								content in serum		
group	Ca g/kg	P g/kg	Cu mg/kg	Fe mg/kg	Zn mg/kg	ADFI[1] g/d	Ca intake g/d	ADG[2] g/d	Cu µmol/L	Fe µmol/L	Zn µmol/L
L	7.92	5.66	12.6	453	36.9	669[a] (± 83.4)	4.89[a] (± 0.61)	397[a] (± 37.6)	27.6 (± 6.09)	36.1 (± 9.06)	5.40[a] (± 1.01)
M	12.7	8.36	13.6	473	37.0	602[ab] (± 79.4)	7.07[b] (± 0.93)	358[b] (± 100)	26.3 (± 6.18)	36.4 (± 5.15)	4.74[ab] (± 1.58)
H	16.9	10.2	12.4	571	39.1	530[b] (± 57.8)	8.36[c] (± 0.91)	192[b] (± 78.2)	30.6 (± 6.04)	28.6 (± 6.78)	3.41[b] (± 0.85)

[a,b,c]indicate significant differences between groups ($p < 0.05$); [1]average daily feed intake; [2]average daily gain

From the 4th week`s trial on piglets of group H showed clinical symptoms of parakeratosis. Especially the areas around the ears, mouth and the medial thighs were severely affected. The lesions did not cause any pruritus. The general condition, the feed intake, and the daily weight gain of group H animals were reduced. The nuclei were histologically retained in the stratum corneum. In some cases the dermis showed lymphocytic infiltrates. The Zn content in serum was the lowest in piglets of group H and significantly different to animals of group L. For the Cu and Fe contents in serum no differences were observed. The daily Ca intake was significantly different in all groups, while piglets of group H showed the highest values.

Conclusion: It is known that high dietary Ca intakes reduce the Zn absorption, because of the competition and displacement for transport systems, especially the divalent cation transporter 1 (2). A compound feed with a Ca content of more than 1% and a Zn content less than 34 mg/kg DM is described as a parakeratogenic diet for swine (3). In this case all diets had approximately similar Zn content, the diet H had even the highest content of 39.1 mg/kg DM. Despite a Zn supply of more than 34 mg/kg DM, the diet H with a Ca content of 1.7% triggered a parakeratosis in the affected piglets and caused the lowest Zn contents in the serum. Piglets of group M did not develop a parakeratosis in spite of a Ca content of 1.3% in the diet. A Ca uptake which is exceeding the requirement, as repeatedly observed in the past, can trigger parakeratosis in piglets regardless a need-based Zn content in the diet. Therefore, it should be critically questioned to what extent the appearance of ear ridge necrosis is associated with high Ca contents in piglet feed.

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28.

Investigations on the influence of a different phosphorus supply on bone′s density and chemical composition in weaned piglets

Untersuchungen zum Einfluss einer unterschiedlichen P-Versorgung auf die Dichte und chemische Zusammensetzung von Knochen bei Absetzferkeln

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The phosphorus (P) supply of pigs is often discussed due to the contribution of high P excretion to eutrophication of soils and water [1]. As P is essential for many physiological processes the extent of possible reduction of this element in compound feeds is limited by reasons of animal health. Especially the skeletal integrity in pigs depends on a sufficient mineral supply. The aim of this study was to generate sensitive diagnostic parameters describing the phosphorus supply of pigs during the flatdeck period from 14.2 kg up to 34.1 kg body weight (bw) at different strategies of P supply.

Methods: Four seven-week-old piglets (mean bw: 16.8 ± 0.73 kg) were euthanised at the beginning of the trial (values at start). Another twelve piglets (mean bw: 14.2 ± 1.07 kg) were allotted to three treatments and kept in groups on straw for 35 days. These three groups were fed botanically and, except P content, chemically identical two phase (>14 kg | >27 kg) compound feeds (based on wheat, barley and soy bean meal) that differed in supplementation with inorganic P (P_i, $NaH_2PO_4 \cdot 2\ H_2O$) and phytase (phyt) *ad libitum* (**C:** P_i +|phyt +, **1:** P_i -| phyt +, **2:** P_i -| phyt -). The diets contained 5.08 | 4.07, 4.02 | 3.46 and 3.72 | 3.83 g P/kg as fed in group C, 1 and 2, respectively (Ca contents: 8.75 | 7.32, 8.23 | 7.71, 8.23 | 7.70 g/kg as fed). The corresponding phytase activities were 1155 | 823, 1071 | 1050 and 521 | 365 FTU/kg as fed.

The left tibiae of all animals were acquired and manually cleaned from the adherent tissue. Subsequently the proximal parts of the tibiae were analysed for their density (Archimedes′ principle) and chemical composition (freeze-dried, extracted with petroleum ether and ashed at 600 °C; Ca and P analysed by atomic absorption spectrometry resp. colorimetrically following microwave ashing). Statistical analyses were done using the SAS® software (LSD-test).

Results: The results of density determination and chemical analyses are shown in Table 1. Significant differences were found in most parameters between treatment C and 1 and all parameters between C and 2 as well as between values at start and treatment 2. No significant differences could be found between values at start and group C.

Table 1: Effects of different P supply on serum P concentrations, bone density and chemical composition of the proximal parts of the tibiae of young pigs (14.2 - 34.1 kg bw), mean ± sd

Parameter		Values at start	Treatment C	Treatment 1	Treatment 2
serum P	[mmol/l]	$1.86^{ab} \pm 0.24$	$2.06^{a} \pm 0.17$*	$1.65^{b} \pm 0.08$*	$1.21^{c} \pm 0.06$*
ash		$414^{a} \pm 11.1$	$426^{a} \pm 20.0$	$376^{b} \pm 17.3$	$335^{c} \pm 10.1$
Ca	[g/kg ff DM[1]]	$142^{ab} \pm 9.53$	$147^{a} \pm 13.4$	$129^{b} \pm 10.1$	$106^{c} \pm 9.46$
P		$72.5^{a} \pm 6.15$	$72.4^{a} \pm 10.1$	$62.7^{ab} \pm 5.38$	$52.9^{b} \pm 4.13$
density	[g/cm³]	$1.1163^{a} \pm 0.0174$	$1.1100^{ab} \pm 0.0215$	$1.0866^{bc} \pm 0.0194$	$1.0780^{c} \pm 0.0119$
ash		$154^{a} \pm 9.81$	$165^{a} \pm 19.7$	$128^{b} \pm 15.2$	$106^{c} \pm 3.36$
Ca	[mg/cm³]	$53.0^{ab} \pm 5.35$	$57.0^{a} \pm 9.34$	$43.9^{b} \pm 6.70$	$33.6^{c} \pm 2.88$
P		$27.0^{ab} \pm 2.88$	$28.2^{a} \pm 5.95$	$21.4^{bc} \pm 3.57$	$16.7^{c} \pm 1.25$

[1]**ff DM**=fat-free dry matter (< 2 % fat);*at the end of the trial; different superscripts indicate significant differences between the treatments ($p<0.05$)

Discussion: Since ash contents in both, ff DM and per volume unit, were lower in pigs of group 1 and 2 compared to values at start, it seemed that either demineralisation in the proximal tibiae occurred or that the newly built bone substance contained distinctly less ash in these two groups. In contrast, treatment C resulted in higher ash contents which seem to reflect the physiological increase in bone ash during growth [2]. These results indicate that ash is a more sensitive diagnostic parameter than bone density in tibiae of weaned pigs and provide data that allow a sound statement on dietary P supply on the basis of blood and bone parameters for young pigs.

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29.

Liver steatosis and tissue iron content in turkeys suffering from hepatic lipidosis

Lebersteatose und Eisengehalt im Lebergewebe unter dem Einfluss der hepatischen Lipidose bei Mastputen

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In consequence of infectious diseases, the iron contents changes in the liver [1]. In the case of hepatic lipidosis in turkeys an infectious aetiology (Picornavirus) is discussed but has not yet been clearly demonstrated so far. Hepatic lipidosis (hlp) of turkeys is characterized by a higher mortality and also by swollen and mottled livers with an excessive accumulation of lipids in the hepatocytes. Hypothesis of this field study was that changes in iron content of the liver in affected animals might indicate and infective pathogenesis.

Methods: The investigations are based on 20 outbreaks of hepatic lipidosis (hlp). Almost exclusively flocks with hens were affected (n=18). The animals were fed a standard commercial fattening diet for turkeys according to their age and official recommendations. From each flock as far as possible at least five livers were collected and analysed on dry matter, crude fat, fatty acids and iron contents. Iron contents were additionally calculated in the fat-free DM. For this purpose, the crude fat content was subtracted from the DM. This was defined as fat-free DM. The livers of hlp animals were categorized concerning their fat content depending on the sum of fatty acid content in the liver (in g/kg DM; fl 1: ≤200, fl 2: >200-250, fl 3: >250-300, fl 4: > 300-350, fl 5: > 350-400, fl 6:> 400-450, fl 7:> 450). For control liver samples from two healthy flocks (c) were taken. The statistical analysis was performed using SAS Enterprise 7.1. One-way ANOVA was done (REGWQ) to compare the results concerning fat categories.

Results: In total 101 liver samples from 22 farms were tested to compare the liver composition of healthy and diseased animals. There were 16 livers from healthy animals and 85 livers from deceased animals from flocks suffering from hepatic lipidosis. The fat content in the liver of affected animals was approximately three times higher than the content in the livers of healthy animals, thus significantly increased (control: 123 ± 36.6^{b}; case: 345 ± 103^{a} g/kg DM). Livers from one stock (at least five liver samples were usually obtained) were always assigned to several fat categories.

Figure 1: Mean levels of iron in the liver tissue (A) and iron in the fat free liver tissue (B) in liver samples of control farms (c; n=16) and affected farms (fl 1 - fl 7; n=85) in dependence of the sum of fatty acid content in the liver (fl 1: ≤200 g/kg DM, n=8; fl 2: >200-250 g/kg DM, n=10; fl 3: >250-300 g/kg DM, n=13; fl 4: > 300-350 g/kg DM, n=10; fl 5: > 350-400 g/kg DM, n=17; fl 6:> 400-450 g / kg DM, n=12; fl 7:> 450 g / kg DM, n=9) ; $p<0.05$.

Conclusion: Iron accumulation in the liver, also known as haemosiderosis, may not always be associated with clinical disease although in severe cases hepatic damage may occur [1]. Increased iron storage in liver tissue related to virus-related liver damage has been described [2]. The high iron load in livers here could indicate that infectious agents are involved in the pathogenesis of hepatic lipidosis in turkeys. The iron contents in the compound diets do not give any explanation for the iron contents in the liver.

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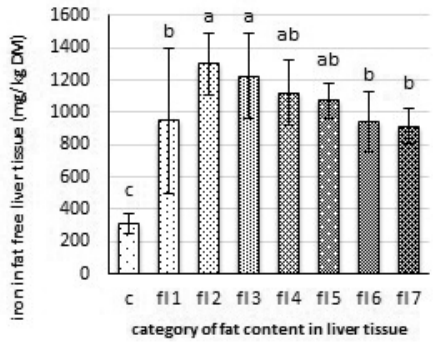

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30.

Effects of dose and source of copper supplementation on quantitative composition of rumen microorganisms

Effekte von Dosis und Quelle bei der Kupfersupplementierung auf die quantitative Zusammensetzung von Pansenmikroorganismen

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Question: The essential trace mineral copper (Cu) has to be supplemented in ruminant diets regarding the reduced bioavailability due to complex-building agents. Otherwise, application of Cu in excess leads to an increased growth performance in piglets because of its antibacterial effects. Therefore, this study investigates the impact of different dietary Cu supplementations (dose, source) on the quantitative composition of ruminal microorganisms.

Methods: Six non-lactating rumen-fistulated cows received 6.5 kg DM (dry matter) of TMR (grass silage, maize silage, wheat meal and soybean meal (solvent-extracted)) in two equal portions per animal and day. Cu-granulate in the form of sulfate ($CuSO_4$) or tri-basic chloride (TBCC) was mixed into the TMR right before feeding. Thus, three levels of total dietary Cu concentrations could be defined: 10 mg/kg DM (recommended supply (1)), 35 mg/kg DM (maximum according to feed law), and 50 mg/kg DM (mild excess). The trial was built up as a Latin square. Throughout 6 experimental periods each of the 6 treatment combinations was applied to every cow. Samples of rumen fluid were collected just before morning feeding (0 h) as well as 1.5 and 3 h after morning feeding. Immediately, per animal 50 ml of rumen fluid were frozen at -20 °C. After lyophilising, the samples were grinded in a mortar and total genomic DNA was extracted. Via real-time quantitative PCR total bacteria, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Streptococcus bovis*, archaea, protozoa and anaerobic fungi were quantified by counting copy numbers of target genes related to 1 g dry matter of rumen fluid. Data were analysed by two-way ANOVA (treatment, animal) followed by contrasts (dose, source) using the GLM procedure of SAS 9.4.

Results: Rising supplementations of Cu did not affect the counts of microorganisms in rumen fluid at all of the three time points except for those of *Streptococcus bovis*. These declined with rising dose of $CuSO_4$ after 1.5 h (log_{10} copy numbers: 7.83 vs. 7.74 vs. 7.66, $p<0.01$) while TBCC remained ineffective. Regarding the proportions of examined microorganisms relative to total bacteria only *Fibrobacter succinogenes* seemed to be affected by Cu supplementation depending on Cu source. The treatment with TBCC showed on average higher values compared to $CuSO_4$ (0 h: 0.73 % vs. 0.61 %, $p=0.10$; 1.5 h: 2.81 % vs. 2.30 %, $p=0.07$; 3 h: 2.98 % vs. 2.45 %, $p=0.05$).

Conclusion: Varying doses and sources of Cu supplementation affected counts of rumen microorganisms only selectively in case of *Streptococcus bovis* and *Fibrobacter succinogenes*. For *Streptococcus bovis* the reductions of counts due to rising doses of $CuSO_4$ do not necessarily indicate a negative impact on rumen function since previous results revealed a concomitant increase in rumen dry matter degradability (2). Altogether, supplementing $CuSO_4$ and TBCC up to mild excess (50 mg/kg DM) does not seem to exert a major harmful impact on rumen microbiota and rumen fermentation potential.

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31.

Mineral levels in grass silages in dependence of harvesting date collected from different regions in Germany

Gehalte an Mengen- und Spurenelementen in Grassilagen in Abhängigkeit des Erntezeitpunktes aus verschiedenen Gebieten Deutschlands

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Silages in general represent the main part of rations for ruminants not only for economic but also for physiological reasons. Therefore, the supply with energy, nutrients and minerals from farm-produced roughages is preferably high. Although chemical compositions of feedstuffs are provided in feed tables, implying a low necessity for silage analyses, it is known that nutrients and especially minerals often display wide variations, which might lead to under- or oversupply when forming rations. Hence, the objective of the study was to determine the range of macro and micro element contents in grass silage samples collected from different regions within Germany, depending of harvest time.

Methods: A total of 246 grass silages were sampled from permanent grassland with dry matter (DM) contents ranging from 161 to 752 g/kg fresh matter. Macro and micro elements were determined after acid hydrolyses by atomic absorption spectrometry (iCE 3500 AAS, Thermo Fisher Scientific GmbH, Germany), whereas phosphorus, sulphur and chloride were measured photometrically, by combustion analysis and potentiometrically (silver nitrate), respectively. The dietary cation-anion difference (DCAD) was calculated according to (1).

Results: Contents of selected macro elements as well as DCAD values in grass silages showed substantial variations (see following table).

cut	n	Ca	P	Na	K	S	Cl	DCAD
		(g/kg DM)						(meq/kg DM)
all[1]	246	6.04[2] (3.13–11.5)	4.04 (0.79–5.43)	2.57 (0.07–6.26)	25.8 (6.88–46.2)	3.09 (0.37–4.75)	10.5 (1.91–31.6)	283 (-88–882)
1st	45	5.53 (3.13–7.66)	4.01 (0.79–5.16)	2.09 (0.07–5.33)	27.1 (6.88–40.5)	2.97 (2.23–4.16)	10.7 (3.29–29.4)	299 (-88–724)
2nd	34	6.61 (3.87–10.8)	3.95 (3.23–4.88)	2.33 (0.07–4.76)	26.0 (16.1–42.4)	3.16 (1.97–4.35)	9.02 (1.91–18.2)	316 (24–882)
3rd	25	6.71 (4.76–9.47)	4.07 (2.70–5.43)	2.81 (0.20–4.97)	25.1 (12.4–46.2)	3.40 (2.35–4.58)	10.3 (5.11–17.9)	263 (-2–737)
4th	12	7.19 (5.16–11.5)	4.13 (3.38–5.09)	3.47 (1.53–5.30)	22.8 (10.1–37.1)	3.68 (2.52–4.75)	8.74 (4.44–14.0)	258 (-88–712)

[1] Samples including silages of known cut (1st to 4th cut) and silages, with missing information on time of harvest.

[2] Mean value and *minimum-maximum*.

Comparatively high contents of sodium in a few samples might be due to the use of chemical silage additives commonly applied as acid anions of organic acids with e.g. sodium as cation. Elevated iron contents in selected samples of up to 13345 mg/kg DM indicate a high degree of soil contamination. An influence of the regrowth seemed to be probable for calcium, sodium and sulphur, where mean values increased with ascending cut number. Variations on DCAD were presumably mainly caused by potassium and chloride showing high coefficients of variation.

Conclusions: Exact knowledge on mineral contents of rations is crucial to meet the animal's requirements and to prevent the occurrence of feed-induced diseases due to deficiency or to excessive intake. In the present study selected mineral contents of the sampled grass silages were not in accordance with tabulated values, reflecting the influence of location, fertilization, crop composition or growth. Special care has to be taken when applying the DCAD concept for dairy cows in the dry period, as DCAD values of grass silages might be considerably under- or overestimated.

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32.

Mineral and trace element contents in pasture grass from areas with or without nature conservation by law characterized by different grazing systems

Mineralstoff- und Spurenelementgehalte im Weidegras von Standorten mit bzw. ohne Naturschutzrelevanz bei Nutzung von unterschiedlichen Beweidungssystemen

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Grassland continues to be the main source of feed for ruminants kept under extensive grazing systems. Particularly with regard to grassland areas with nature conservation only in few cases both the mineral and trace element contents of pasture grass are determined. Therefore deficiencies in animal supply during long periods of grazing may arise. The objective of the present study was to give an overview about mineral and trace element contents of different extensive pastures areas and pasture management systems for sheep.

Methods: 147 pasture grass samples deriving from 34 grassland locations (23 high nature value area (§ 32 State nature conservation law of Baden-Württemberg or FFH-directive) and 11 without nature conservation by protection of law) from 18 sheep farms (12 transhumance grazing systems (1) and 6 fenced off grazing) were analysed. Fresh grass samples were pre-dried (32 h/60°C) and ground (1 mm mesh size), followed by dry matter (DM) determination using near infrared spectroscopy (FOSS 5000). For mineral and trace element determination samples were digested in a microwave pressure digestion system (HNO_3/H_2O_2) and analyses were performed with an inductively coupled plasma optical emission spectrometer (ICP-OES) according to DIN EN 15510:2007. Means were compared by t-test.

Results: The mineral composition of pasture grass with or without nature conservation differs largely within the individual elements (Table). On average contents of P (2.0 and 2.7 g/kg DM) are as low as expected for unfertilized grassland (except sheep excrements). In particular mean values of Na with 0.07 and 0.04 g/kg DM show a very low level and only average contents of Ca, P, Mg, Mn and Fe would fulfil maintenance requirement of sheep. Noteworthy are the high average and maximum values of Mn and Fe for both types of grassland. Transhumance grazing with keeping sheep in pens out of the pasture overnight results in lower Ca, P and K concentrations in the pasture grass compared with fenced off grazing.

	Pasture grass with nature conservation by law (n=98)				Pasture grass without nature conservation by law (n=49)			
Item	Mean	Min	Max	SD	Mean	Min	Max	SD
Dry matter (DM)(g/kg)	297[a]	159	462	64	235[b]	122	357	56
Calcium (Ca) (g/kg DM)	7.5[a]	1.3	13.1	1.3	7.4[a]	2.9	14.2	2.5
Phosphorus (P) (g/kg DM)	2.0[a]	0.7	5.1	0.9	2.7[b]	1.0	5.9	1.0
Magnesium (Mg) (g/kg DM)	1.9[a]	0.8	4.4	0.7	1.9[a]	1.1	2.5	0.4
Potassium (K)(g/kg DM)	16.8[a]	7.4	31.0	4.7	22.7[b]	11.5	37.4	6.6
Sodium (Na) (g/kg DM)	0.07[a]	n.d.[1]	1.2	0.2	0.04[b]	n.d.[1]	0.2	0.03
Zinc (Zn)(mg/kg DM)	31[a]	14	79	12	34[b]	19	62	10
Manganese (Mn) (mg/kg DM)	163[a]	26	1333	209	195[b]	44	708	166
Copper (Cu)(mg/kg DM)	5.1[a]	3.2	8.5	1.1	5.9[b]	3.1	10.8	1.8
Iron(Fe)(mg/kg DM)	193[a]	56	712	136	218[b]	34	890	220

[1]n.d. (below limit of quantification), [a,b] within a line with $P < 0.05$ significant differences of means

Conclusion: The mineral and trace element contents of the investigated sheep pastures differ widely from those of permanent pastures. This should be taken into account both for mineral supplementation of sheep in order to meet their requirements and for the calculation of nutrient balances for sheep farms.

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33.

Copper supply of sheep in Mecklenburg-Western Pomerania - current state

Aktueller Stand der Kupferversorgung von Schafen in Mecklenburg-Vorpommern

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Copper (Cu) is an essential nutrient, which is important for many functions, including skeletal health, formation of the nervous system, fertility and red blood cells' production. If the Cu supply of sheep is insufficient, consequential damages of the lambs (e.g. sway back) might appear. If there is an oversupply, Cu has toxic effects (e.g. jaundice, 1). Depending on the type of housing and feeding, an adequate Cu supply represents still a problem in sheep feeding (2). The aim of this study was to get an overview of the Cu feed intake and Cu content in the liver of sheep under usual feeding conditions in Mecklenburg-Western Pomerania (MWP).

Methods: In MWP 25 sheep farms were selected, that differed in farming and feeding methods. At 10 farms, the sheep were kept exclusively indoors. Sheep feeding was conducted on 5 farms with (*barn, mineral* +) and on 5 farms without any mineral feed (*barn, mineral* -). Of 15 pasture holding farms, 10 farms supplemented mineral feed (*pasture, mineral* +) and 5 farms did not (*pasture, mineral* -). The feed intake was calculated on the base of intake capacity of 2% of live weight. Cu contents in feed and in hepatic tissue (4 livers per farm) were analysed by common methods of VDLUFA. A classification of hepatic Cu concentration according to (3) was used: undersupply (< 200 mg/kg liver DM), adequate supply (200 mg/kg liver DM), oversupply (> 200 mg/kg liver DM), toxic (> 600 mg/kg liver DM). Data were analysed using the statistic program SPSS version 20.

Results: Within the barn holding farms, mineral supplementation indicated differences ($p < 0.05$) in Cu intake and Cu content of the liver as shown in the following table.

feeding regime	n	feed intake (FI) (mg Cu/kg DM)	n	liver tissue (LT) (mg Cu/kg DM)	correlation Cu-FI/Cu-LT (R^2)
pasture, mineral +	10	25.8[a] (± 13.0)	40	350[a] (± 176)	0.398
pasture, mineral -	5	22.9[a] (± 5.20)	20	366[a] (± 221)	0.686
barn, mineral +	5	43.4[a] (± 12.3)	20	627[a] (± 58.6)	0.297
barn, mineral -	5	17.1[b] (± 3.39)	20	179[b] (± 33.9)	0.675

[a,b] indicate significant differences within farming method and between feeding method ($p < 0.05$), DM = dry matter

This influence was not confirmed for pasture holding farms. The correlation of Cu intake to Cu content in the liver was higher without mineral supplementation than with. However, Cu intake shows merely the current state, however the Cu content in the liver is marked long-lasting. The real evidence of correlation needs to be critically examined. All farms showed an oversupply of dietary Cu, in one case even intoxication occurred. Only in sheep of the farms "barn, mineral -" Cu levels of the liver indicate an insufficient intake.

Conclusion: This study demonstrated that the continuous need-based Cu supply is problematical, especially for pasture feeding. As well, the use of mineral feed is not adequate. If consuming sheep liver the Cu intake is generally high. With the present values, the intake of 100 g liver (FM) would already exceed by twice the Tolerable Upper Intake Level for humans of 5 mg/d (4). For a proper Cu supply and for animal health but also food safety, it is necessary to know the dietary content of Cu in the used feed. Moreover, the content of antagonistic elements (molybdenum, sulphur, zinc) must be considered. Especially the continuous Cu supply (seasonal variations in the pasture growth) should be assured by regular feed analyses.

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34.

The adequate copper supply of sheep - still a challenge? An evaluation of liver samples

Zum Cu-Gehalt in Schaflebern - eine Auswertung von Feldproben

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Copper (Cu) has diverse functions as an apoenzyme and as a part of structural and transport proteins. Sheep are known to be highly susceptible for a Cu intoxication, as the tolerance level is lower than in many other species. Thus, analysis of Cu content in liver of slaughtered/fallen animals has become a common method to monitor Cu supply on herd level, as Cu contents of roughage can vary widely and supplementary feed are often Cu-free. Chronic Cu intoxication is a feared problem, but Cu deficiency might occur just as often (1). Liver samples, which were submitted between 2012 and 2016 to the Institute for Animal Nutrition (Univ. of Vet. Med. Hannover, Foundation), were evaluated. The hypothesis of the study was that there are still cases of oversupply as well as of deficiency in the field.

Material and Methods: Liver samples of sheep were sent to the mentioned service laboratory regularly by animal owners or from regional authorities for further diagnostics (necropsy samples). Detailed case reports were usually not given and often the Cu content was the only requested parameter. Origin of samples was mainly North Germany. Cu contents were analysed after "wet ashing" by using an atomic absorption spectrometer, whereby values in liver from 200-500 mg Cu/kg DM ("reference values") were stated as common for adult sheep (2). Lambs are known to have lower values, but the liver of newborns already contains 50 mg Cu/kg DM (3).

Results and Discussion: The following table presents Cu contents in liver of adult sheep:

Year	2012	2013	2014	2015	2016
n (number of analyses)	33	31	45	41	49*
Cu content, mg/kg DM**, median	149	255	72.0	138	147
Cu content, mg/kg DM, first quartile	40	28.8	24.3	49.3	55.2
Cu content, mg/kg DM, third quartile	232	821	193	269	373
Samples < "reference values" (%)	66.7	45.2	77.8	65.9	55.1
Samples > "reference values" (%)	0.660	38.7	2.22	7.32	14.3

* considering all analysed samples until end of October 2016; ** DM = dry matter

There was a wide range of variation of Cu content in sheep liver. For the last 4 years, liver samples of adult sheep representing "normal levels" only amounted to 16.1-32.7 % of all samples. The proportion of samples below "normal values" is worrying, as a deficiency in Cu supply might be easily prevented by mineral supplements. Regarding hepatic tissue of lambs, 20.0-33.3 % (in the years 2012-2016) of all samples had Cu contents below the minimum expected value of at least 50 mg/kg DM.

Conclusion: It is recommended to check regularly the Cu levels of sheep liver. Supplementary feeds often do not contain Cu and Cu contents of green fodder vary depending on soil and fertilizers used. Finally there are cases of "secondary Cu deficiencies" due to sulfur/molybdenum interactions but also due to an excessive iron intake (repeatedly observed in cattle fed grass silages with high iron contents). Testing Cu contents in liver gives quantitative information on Cu accretion and might be often more useful than calculating/analysing the dietary Cu content. Here, a Cu deficiency had a higher prevalence than an overdosing. It has to be considered that there was no random choosing system but a monitoring of "cases". Other actual studies (4) showed that in other parts of Germany Cu levels in sheep liver can be quite high, underlining the importance of a regular control.

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35.

Can *ex vivo* tissue models be used to evaluate the effects of endotoxins on livestock animals?

Können ex vivo Gewebemodelle verwendet werden um Effekte von Endotoxinen bei Nutztieren zu evaluieren?

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Endotoxins, also called lipopolysaccharides (LPS), are constituents of the outer membrane of Gram-negative bacteria. During bacterial imbalance, endotoxin concentration in the gut can rapidly increase. The knowledge about the endotoxin concentration in the intestine of livestock animals, however, is very limited. Nevertheless, it is known that during rumen acidosis, endotoxin concentration in the rumen can increase up to five times (1). Furthermore, endotoxins can enter the blood stream through an impaired gut or rumen barrier. LPS can thereby have an influence on the gastrointestinal health in various species, e.g. pigs (2,3) or horses (4), leading to inflammation processes. Furthermore, endotoxins are also discussed to be involved in the pathology of several other diseases such as laminitis in ruminants (5) and horses (6). We therefore investigated *ex vivo* models to evaluate the influence of endotoxins on porcine intestinal tissue, bovine and equine hoof tissue.

Methods: Porcine jejunal explants were cultivated in 12 well plates with 1.5 mL cultivation medium at 39 °C and 5% CO2. The water soluble tetrazolium (WST)-1 assay was used to evaluate viability of explants after 2, 4, and 24 hours. The explants were furthermore stimulated with 1, 10, and 100 µg/mL of LPS for up to eight hours. After stimulation, explants were frozen in liquid nitrogen and stored at -80 °C. Gene expression of toll-like receptor 4 (TLR4), Interleukin 6 (IL-6), Interleukin 8 (IL-8) and tumor necrosis factor (TNF) was evaluated via RT-qPCR. In addition, claw and hoof explants, consisting of three layers: the inner hoof wall, epidermal lamellae and connective tissue, were cultivated in 24 well plates with 1 mL cultivation medium at 37 °C and 5% CO2. Viability was assessed as well with the WST-1 assay after incubation. LPS was added to the bovine [0, 1, 10, 100 µg/mL] and equine [0, 2.5, 10, 100 µg/mL] explants. Tissue integrity of explants was measured with a calibrated force transducer (= separation force) as described by Reisinger *et al.* (6).

Results: Viability of intestinal porcine explants was significantly decreased after 4 hours. LPS significantly increased the expression of TLR4 at 2 hours stimulation, but had no effect on expression of IL-8. However, there was a significant increase of IL-6 at 2 hours and a significant increase of TNF at 2 and 4 hours. Viability of hoof and claw explants was not affected after 24 hours incubation. There was no effect of 1 µg/mL LPS on tissue integrity of bovine claw explants. However, at higher concentrations [10 and 100 µg/mL] LPS significantly decreased the separation force in bovine explants by 50 and 65%, respectively. In equine hoof explants there was no effect of 2.5 µg/mL LPS. Higher concentrations of LPS [10 and 100 µg/mL] led to a significant reduction of separation force by 45% and 49%, respectively, as well.

Conclusion: Endotoxins had a negative effect on porcine intestinal explants as well as on bovine and equine hoof explants. Interestingly, similar concentrations of LPS led to negative effects on both tissue types. This might be explained by the different design of the *ex vivo* models, e.g. thickness of the tissue, incubation time, leading to a similar sensitivity. As alternatives to animal testing are highly eligible, *ex vivo* cultivation of explants might provide an alternative tool to investigate the role of endotoxins during intestinal inflammation in pigs as well as laminitis in ruminants and horses. Furthermore, the presented model using claw and hoof explants can be used to test further potential trigger factors of laminitis and the interaction between endotoxins and these factors.

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36.

Examination and evaluation of the biomarker Sa/So (sphinganine/sphingosine) ratio in pigs after i.v. and oral application of fumonisin B1 (FB1) with or without a feed additive with fumonisin esterase activity

Untersuchung und Evaluierung des Biomarkers Sa/So (Sphinganin/Sphingosin) Ratio bei Schweinen nach i.v. oder oraler Gabe von Fumonisin B1 (FB1) mit oder ohne Zusatz eines Futtermittelzusatzstoffes mit Fumonisinesterase-Aktivität

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Fumonisins, particularly FB1, are mycotoxins derived predominantly from *Fusarium*-contaminated maize. These toxins have been associated with immune-modulation and organ specific alterations in pigs depending on dose and duration of exposure (1). In the present study the efficacy of a feed additive with fumonisin esterase activity for detoxification of FB1 was investigated, using the sphinganine/ sphingosine ratio as a potential biomarker for fumonisin exposure.

Methods: Thirty-one barrows (34.4 ± 2.7 kg BW) received a control diet during an adaptation period and were surgically equipped with permanent indwelling jugular catheters. Thereafter, pigs were housed in metabolism crates and received one of five treatments: CON (control diet), FB1 i.v. (CON and 100 µg FB1 i.v./kg BW), HFB1 i.v. (CON and 56.2 µg HFB1 i.v./kg BW), FUM oral (120 mg FB1/kg diet), FUM + enzyme (120 mg FB1/kg diet and 240U FUM*zyme*®/kg feed). HFB1, a metabolite of FB1 without known biological activity, was used as a negative control. A 120-hours sampling period started after single dosing, during which frequent blood samples (0h, 1h, 2h, 3h, 3.5h, 4h, 6h, 8h, 12h, 24h, 48h, 72h, 96h, 120h) were taken. Pigs were sacrificed after 120h and *liquor cerebrospinalis* was sampled. Sphinganine (Sa) and sphingosine (So) were analysed in serum and liquor and their ratio Sa/So was calculated. Data were statistically analysed with PROC MIXED in SAS with group, time and Sa/So ratio as main factors as well as their interactions and 0h values as covariable.

Results: After single dose treatment, a first increase of Sa/So ratio in blood could be detected after 12 hours in the FB1 i.v. group ($p<0.001$) and reached significance for both FB1 groups (i.v. and oral) after 24 hours (data not shown). Furthermore, no significant elevation of the biomarker in blood was detected in CON, HFB1 i.v. and FUM + enzyme groups.

This demonstrates on the one hand that, in contrast to FB1, the hydrolysis product HFB1 does not have a negative impact on the sphingolipid metabolism, which is the basis for fumonisin toxicity. On the other hand, it was shown that FUM*zyme*® is able to hydrolyse fumonisins *in vivo*.

In liquor samples, Sa/So ratio reflected the situation in serum, being significantly higher for FB1 i.v. and FUM oral groups, whereas CON, HFB1 i.v. and FUM + enzyme groups were not altered.

Conclusions: The Sa/So ratio appears to be a suitable biomarker for fumonisin exposure and potential detoxification methods of the mycotoxin. Using a fumonisin esterase as feed additive was proven to be an effective tool in preventing the adverse fumonisin effects as indicated by an unaltered Sa/So ratio.

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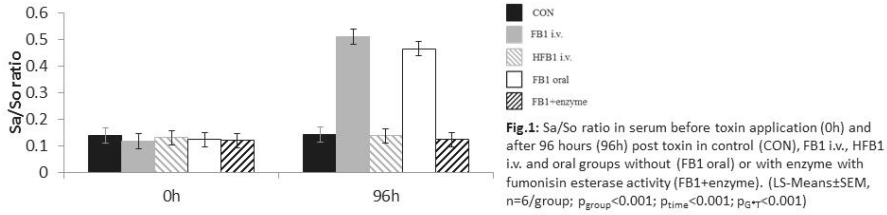

Fig.1: Sa/So ratio in serum before toxin application (0h) and after 96 hours (96h) post toxin in control (CON), FB1 i.v., HFB1 i.v. and oral groups without (FB1 oral) or with enzyme with fumonisin esterase activity (FB1+enzyme). (LS-Means±SEM, n=6/group; $p_{group}<0.001$; $p_{time}<0.001$; $p_{G*T}<0.001$)

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37.

Effect of glyphosate residues in animal feed on ruminal fermentation in dairy cows

Einfluss von Glyphosatrückständen in Futtermitteln auf die Pansenfermentation bei Milchkühen

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Glyphosate (N-phosphonomethylglycine) is a broad-spectrum, non-selective herbicide and the most used agent in agriculture worldwide for weed control and plant growth regulation. In vitro studies showed effects on ruminal bacteria and protozoa occurring in the rumen. Therefore, the objective of the present study was to investigate the effect of glyphosate on ruminal microbial crude protein synthesis (MCP) and fermentation in dairy cows.

Methods: Eigth pluriparous lactating German Holstein cows fitted with rumen and duodenum cannulae were used in an experiment lasting for 17 weeks. In week 0 all cows received a total mixed ration (TMR) consisting of 30% maize silage, 30% grass silage, 40% concentrate (on a dry matter basis) for *ad libitum* consumption. During week 1 until week 16 cows were divided into two groups fed *ad libitum*, either an uncontaminated control TMR (CON) or a glyphosate contaminated (treatment according to the legal regulations during plant cultivation) TMR (GLY). The TMRs were composed of 21% maize silage, 42% grass silage, 30% concentrate and 7% straw (on a dry matter basis). The average glyphosate intake of the CON cows was 0.60 mg/d and of the GLY cows 62 mg/d. In week 8 samples of duodenal chyme were taken every ten hours for five consecutive days and pooled over the sampling period according to von Soosten et al 2016 (1). For calculation of the daily duodenal dry matter flow (DMF) Cr_2O_3 was used as a marker. The microbial N fraction of the duodenal non-ammonia N was estimated by NIRS (2) to calculate daily MCP. The fermented organic matter (FOM) in the rumen, utilizable crude protein (uCP), undegradable crude protein (UDP) and ${}_aNDF_{om}$ digestibility were calculated according to equations presented by Aschemann et al. (3). Data were analysed by using STATISTICA software version 12.

Results: No effects of glyphosate residues in animal feed on MCP were observed. The FOM (% of OM intake) in the rumen was similar in both groups. The microbial efficiency expressed as MCP per kg FOM remained unchanged. The amount of UDP at the duodenum related to crude protein intake and uCP flow at the duodenum were not influenced. The digestibility of ${}_aNDF_{om}$ was not affected. All data are presented in Table 1.

Effect of glyphosate residues on FOM, ${}_aNDF_{om}$ digestibility, MCP, protein degradation and uCP (Values presented as means ± standard deviation)

	CON (n = 4)	GLY (n = 4)	p-value
MCP (g/d)	1340 ± 312	1355 ± 158	0.943
FOM (% of OM intake)	54 ± 5	55 ± 7	0.801
MP/FOM (g/kg)	180 ± 5	171 ± 30	0.633
UDP (% of CP)	25 ± 5	22 ± 2	0.312
uCP (g/d)	1800 ± 387	1784 ± 182	0.949
${}_aNDF_{om}$ Digestibility (%)	56 ± 10	60 ± 10	0.672

MCP=microbial crude protein; FOM=fermented organic matter; UDP=undegradable crude protein; uCP=utilizable crude protein; ${}_aNDF_{om}$=neutral detergent fibre

Conclusion: In the present study glyphosate residues in animal feed showed no influence on microbial protein synthesis and nutrient flow into the duodenum as well as protein degradation. However, effects on ruminal microbial community cannot be excluded and will be investigated.

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38.

Contents of cadmium and lead in grass silages from different cuts and regions in Germany

Gehalte an Cadmium und Blei in Grassilagen verschiedener Erntezeitpunkte und aus verschiedenen Gebieten Deutschlands

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Heavy metals enter plants via diverse pathways, but accumulation is generally influenced by characteristics of the soil like e.g. pH value and content of organic matter. Whereas cadmium (Cd) is predominantly taken up by the roots, lead (Pb) levels depend mainly on soil contamination of roughages. Flooded areas might especially be affected, so that the question occurs if statutory critical values in feedstuffs are exceeded. Hence, the objective of the study was to determine the range of Cd and Pb contents in grass silage samples collected from different regions within Germany.

Methods: A total of 242 grass silages (9 originated from flooding areas; different cuts from the same location) from permanent grassland were sampled with dry matter (DM) contents ranging from 161 to 752 g/kg fresh matter. The Cd and Pb contents were determined after acid hydrolyses by atomic absorption spectrometry (iCE 3500 AAS, Thermo Fisher Scientific GmbH, Germany, detection limit 0.008 and 0.2 ppm/kg for Cd and Pb, resp.) on a basis of 88% DM. Results are given as frequency distributions (in %): class I (Cd <0.1 ppm; Pb <1 ppm), class II (Cd 0.1-0.5 ppm; Pb 1-5 ppm), class III (Cd >0.5-1 ppm; Pb >5-30 ppm) and class IV including values exceeding the upper limits in silages according to the directive 2002/32/EG for Cd (1 ppm) and Pb (30 ppm), respectively.

Results: Frequency distributions (in % of total sample number) of content classes of Cd and Pb (in ppm) in grass silages are shown in the following table. From all silages 9 samples (corresponding to 4% of 242 samples) exceeded the allowed upper Cd value of 1 ppm (class IV, 5 samples from flooding areas), where contents as high as 2.44 ppm (2nd cut) and 2.45 ppm (1st cut) were found.

cut	n	class I		class II		class III		class IV[2]
		Cd (<0.1)	Pb (<1)	Cd (0.1-0.5)	Pb (1-5)	Cd (>0.5-1)	Pb (>5-30)	Cd (>1)
all[1]	242	9[3] (0-0.08)[4]	11 (0-0.99)	56 (0.11-0.50)	64 (1.04-4.95)	31 (0.52-0.98)	25 (5.10-14.6)	4 (1.01-2.45)
1st	44	7 (0-0.02)	20 (0-0.93)	66 (0.13-0.50)	57 (1.38-4.73)	23 (0.58-0.95)	23 (5.18-14.6)	5 (1.04-2.45)
2nd	34	15 (0-0.08)	6 (0-0.04)	38 (0.11-0.47)	59 (1.10-4.77)	32 (0.55-0.84)	35 (5.35-13.2)	15 (1.01-2.44)
3rd	25	24 (0-0.02)	20 (0-0.99)	44 (0.20-0.50)	48 (1.43-4.66)	28 (0.54-0.98)	32 (5.57-7.56)	4 1.01
4th	12	0 -	0 -	58 (0.22-0.46)	58 (1.27-4.87)	42 (0.52-0.86)	42 (6.37-8.89)	0 -

[1]Samples including silages of known cut (1st to 4th cut) and silages, where information was not available.
[2]No samples found for content class IV of Pb (> 30 ppm). [3]Frequencies in %. [4]*Minimum-maximum* within one class.

Likewise for Cd, most silages were found in content class II for Pb (1-5 ppm), followed by class III (5-30 ppm) with a maximum value of 14.6 ppm (1st cut silage, same sample with Cd content of 2.45 ppm, see before). The maximum set Pb content of 30 ppm was not exceeded in any sample.

Conclusions: Despite the fact that 65% of all analysed samples showed Cd contents of less than half of the statutory maximum content (content classes I and II), Cd contamination of roughages still seems to be an issue regarding elevated values of individual samples. In contrast to Cd, the critical value for Pb (>30 ppm) might be rarely reached, as 75% of all grass silage samples showed contents of <5 ppm (content classes I and II). Although high Cd contents corresponded with high Pb values in selected samples, no correlation was found between these both elements (R^2=0.06, ± s=2.72, n=242) in general.

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39.

A case of ryegrass staggers (intoxication with Lolitrem B) after feeding straw from the grass seed production to horses

Fallbericht: "Taumelkrankheit" aufgrund einer Vergiftung mit Lolitrem B nach der Fütterung von Grassamenstroh in einem Pferdebestand

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Straw from the production of grass seeds is not seldom used as an alternative to hay in horse feeding. Grass straw is used as a roughage low in energy that is less lignified than "normal" straw. But more often the lower costs are at least one reason to use grass straw instead of hay.

Methods: In August 2012 in a riding center housing 50 horses, initially 4 horses out of a group of 8 showed diarrhea. Two days later 6 horses from this group and 3 from another group showed ataxia. Over the day the horses were kept either on a sand paddock or a barren pasture with an additional offer of "hay" from the grass seed production. A new batch of grass straw was introduced 2 days prior to the occurrence of the first symptoms. Other horses housed in the same facility that were not fed with grass straw showed no comparable clinical signs. After shifting from the grass straw to a common hay all signs of illness disappeared within one week. Based on the clinical signs a representative sample of the grass straw taken from the bales actually fed to the affected horses underwent sensory evaluation and was further analyzed for lolitrem B (HPLC).

Results: The yellow-green sample only consisting of *Lolium perenne* showed a bulky grip due to a high proportion of stems; the grass seed heads were mostly threshed out. The sample had an inconspicuous and flat odor and did not show any signs of deterioration by bacteria or molds. HPLC analysis revealed a content of 3.5 mg lolitrem B/kg grass straw.

An intoxication with lolitrem B results in neurological signs like tremor and ataxia but also gastrointestinal disturbances, increased blood pressure and heart rate and can be noticed after the ingestion of hay with concentrations of 1.2 mg lolitrem B/kg or more [1]. Lolitrem B is produced by *Neotyphodium lolii*, a symbiotic endophyte in perennial ryegrass making it more resistant against droughts and several insects [2]. *N. lolii* is endemic in many parts of Europe. Moreover, artificially endophyte infected cultivars are used in the production of grass seeds for lawns, playgrounds, etc. Toxin concentrations in the grass/hay depend largely on weather and vegetation stage, with higher concentrations after hot and dry summers as well as in older plants. Further, *N. lolii* not only produces the tremorgen lolitrem B but also ergovalin (prolactin-antagonist, vasoconstrictive) and peramine (insect-deterring properties). While sheep and cattle are relatively resistant to lolitrem B and signs of intoxication ("ryegrass staggers") are first seen when concentrations exceed 2-2.5 mg/kg DM [3], horses are much more sensitive. Next to ryegrass staggers further intoxications via grass/hay due to endophytes producing several ergot alkaloids (e.g. *Neotyphodium coenophialum*) are possible. Because infected plants have growth advantages under stressful conditions and endophytes are mainly distributed by the seeds, the rate of infected plants within a population can increase over years. Therefore grass, silage or hay harvested from these sites can become toxic for horses and cattle over time. As differential diagnosis for this case other tremorgens like janthirem B which is produced by *Penicillium* ssp. growing on dead plant material in ryegrass pastures should be considered.

Conclusion: Depending on the clinical signs (diarrhea and ataxia) and the detection of lolitrem B in considerable concentrations in the grass straw fed to these horses make it reasonable to assume an intoxication caused by lolitrem B. These intoxications are commonly observed in early autumn, shortly after harvest of the grass, as during longer storage of grass straw the lolitrem B concentration decrease due to bacrterial degradation. Clinical signs of lolitrem B intoxications provide a risk for secondary injuries due to ataxia and imbalance, but generally they are completely reversible when the contaminated grass straw is removed from the diet. As there is little knowledge about the possible occurrence of toxins like lolitrem B or ergovalin in ryegrass, corresponding diagnoses are rare.

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40.

Antidotal efficacy of free methionine in broilers fed a basal diet in which corn was incrementally substituted by cassava chips

Die antidotale Wirksamkeit von freiem Methionin bei Broilern, die mit einer basalen Diät versorgt wurden, in der Mais schrittweise durch Maniokspäne ersetzt wurde

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Methionine requirement is mainly governed by its metabolic needs in protein synthesis. Methionine partitioning also include metabolic detoxification primarily because its structural sulphide sulphur (-SCH_3) is a precursor of mercaptan group (R-SH). Mercaptan has a potent affinity to degrade polar charged feed toxicants in addition to being a component of glutathione. Thus dietary methionine should be optimised in support of feed safety and nutritive value when feeding challenging ingredients such as cassava. Cassava harbours HCN (cyanide) in its free and precursor forms (cyanogenic glucosides). Cyanide is a charged cytotoxic agent which binds cytochrome c oxidase leading to disruption of the electron transport chain, ultimately blocking cell respiration and ATP production. To neutralise cyanide the body primarily uses mitochondrial rhodanese enzyme pathway where mercaptan degrades cyanide to thiocyanate (SCN) (Rosling, 1994). Apart from cyanide exposure cassava based diets risk protein and amino acid deficiencies since these nutrients exist at near zero levels in cassava. Whether methionine is able to bridge these gaps is still unclear. Thus increasing levels of cassava chips (CC) in replacement of corn were titrated in broilers at moving methionine supplementation.

Methods: A least cost basal diet containing 590 g/kg corn at starter and 710 g/kg corn at finisher phases was formulated to contain standard energy and nutrient levels. Then CC was incorporated at 0, 25, 50, 75 and 100 % of corn. Relative to the basal diet CC inclusion resulted in severe reduction in CP, M+C, lysine and ME. Methionine as DL-Methionine was supplemented at 0, 1 or 2 g/kg diet. The resulting 15 pelleted diets were completely randomly assigned to triplicates of 10 d old unsexed Arbor Acre plus broilers and fed *ad libitum* for 56 days. All animal procedures were approved by animal welfare regulators and adhered to the guide for the care and use of agricultural animals in research and teaching (FASS, 2010). Performance data and serum SCN were modelled by regression and means compared at $P < 0.05$.

Results: Analyzed total and free HCN in the cassava chips fed were 377 mg/kg and 20 mg/kg respectively. A curvilinear depression was observed in BW gain and fcr as birds consumed more cassava without free DL-Met (Figure 1). These confirmed the dietary ME and protein limitations and probably cyanotoxicosis as cassava ingestion increased. Methionine however corrected these pathotoxicological conditions to a curvilinear ascending polynomial in BW gain or to a linear improvement in fcr as birds ingested more cassava plus 1 g/kg DL-Met. But increasing crystalline methionine density to 2 g/kg diet significantly depressed BW gain and fcr ($P < 0.05$), when cassava inclusion was beyond 50% of basal corn level (Figure 1). Serum SCN (µg/mL) did not respond to CC level at 0 g/kg DL Met, suggesting that without additional methionine supplementation cyanide detoxification mechanisms were limited (data not shown). At 1 g/kg DL-Met however, serum SCN was statistically ($P > 0.05$) or numerically greater than all other treatments when cassava was used at 75% or 100%, respectively. On the other hand the velocity of cyanide detoxification to SCN was limited when 2 g/kg DL-Met was supplemented.

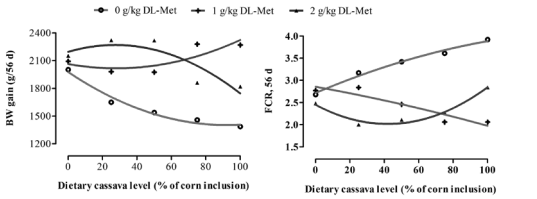

Figure 1. Paths of broiler performance in dependence on dietary cassava chips and DL-Methionine supplementation

Conclusion: We conclude that methionine is efficacious to upgrade cassava fully to the nutritive status of corn under the study conditions.

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41.

Investigations on praecaecal digestibility of a home-made food based diet for patients with exocrine pancreatic insufficiency - studies in ileo-caecal fistulated pigs

Untersuchungen zur praecaecalen Verdaulichkeit einer home-made auf Lebensmitteln basierten Ration für Patienten mit exokriner Pankreasinsuffizienz – Untersuchungen an Schweinen mit ileo-caecaler Umleitungskanüle

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Exocrine pancreatic insufficiency (EPI) is a disease occurring in humans as well as veterinary patients causing maldigestion and malabsorption. The pancreatic enzyme replacement therapy (PERT) is standard worldwide but there are also dietary recommendations regarding the use of highly digestible compounds to prepare a home-made diet for dogs (1). The EPI is well known in canine medicine – and beside PERT the use of eggs, curd cheese and cereals is recommended (1). This study aimed to test the praecaecal digestibility of a home-made diet – using foodstuffs being highly digestible in healthy individuals – to test, whether enzyme therapy is needed in EPI patients under these conditions. The pancreatic duct ligated pig (PL-pig) was used as an established model for EPI.

Material and Methods: The study was performed in 8 adult female minipigs (Ellegaard). All animals were fitted with an ileo-caecal fistula. In 4 pigs (PL-pigs) the pancreatic duct was ligated to induce an EPI. The test diet consisted of 250 g of culled cheese (20 % fat), 100 g of oat flakes, 75 g full egg powder, 25 ml of olive oil, 9.88 g of methylcellulose and 500 g of milk (3.5 % fat). The praecaecal (pc) disappearance rate (DR) of dry matter (dm), crude fat (cfa), crude protein (cp) and starch (st) was tested in an established screening test model according to (2). The PL-pigs underwent the trial twice – with (+ 300.000 IU of lipase, 17.332 IU protease, 306.455 IU amylase per meal) and without PERT. Samples of ileal chyme were freeze dried and standard methods according to (3) were used to determine nutrient content in diet and ileal chyme; starch was analysed polarimetrically while chromium oxide was analysed according to (4). Student`s t-test was used for statistical analysis to compare Control and PL resp. PL + PERT while pair t-test was used to compare effect of PERT (PL vs. PL + PERT) by using SAS®.

Results: The home-made diet showed very high pc DR in healthy controls (showing values of 93.5 % for fat and 99.3 % for starch). In PL-pigs receiving no PERT the pc DR was much lower for dm, cfa and cp – while for starch a high pc DR above 90 % was detected – but still differing significantly from the values observed in controls. PERT resulted in a significant increase of pc DR values – levels of controls were reached for cfa and st.

Table 1: Praecaecal disappearance rate (%) of dry matter and nutrients in healthy controls and PL-pigs (without/with PERT) fed a home-made diet based on foodstuffs supposed to be highly digestible

	Praecaecal disappearance rate (%)			
	Dry matter	Crude fat	Crude protein	Starch
Control	82.1 ± 1.16a	93.5 ± 0.653a	82. 8 ± 2.60a	99.3 ± 0.127a
PL	39.9 ± 8.84b*	26.3 ± 23.8b*	18.5 ± 2.17b*	94.4 ± 5.92a*
PL + PERT	77.2 ± 1.46b#	91.9 ± 1.55a#	71.6 ± 3.67b#	98.4 ± 0.629a*

Different letters mark significant differences ($p<0.05$) to control group while different symbols mark significant effect of PER

Conclusion: Although the foodstuffs used in this home-made diet were supposed to be highly digestible the PL-pigs without PERT showed a markedly lower pc DR of cfa and cp. The finding that the pc DR of the nutrients was distinctly reduced in PL-pigs – although the compounds used were selected for high digestibility rates – underlines the need for PERT in patients suffering from EPI. Furthermore it shows clearly that the digestibility of feedstuffs cannot be estimated in any case of maldigestion by taking into account the values observed in healthy individuals.

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42.

Effect of experimentally-induced exocrine pancreatic insufficiency on the intracellular cobalamin status in pigs

Die Auswirkung einer experimentell induzierten exokrinen Pankreasinsuffizienz auf den intrazellulären Kobalamin-Status beim Schwein

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Dietary cobalamin (vitamin B_{12}) absorption, which takes place primarily in the ileum, and its conversion to intracellularly active co-enzymes requires many physiological steps including intestinal uptake by an intrinsic factor (IF)-mediated transport and processing as well as intracellular release and compartmentalization to cobalamin-dependent enzymes (1). Receptor-mediated endocytosis of cobalamin occurs exclusively in the ileal mucosa due to the presence of an IF-cobalamin complex. IF is mainly synthesized by gastric parietal cells (GPCs) in humans and by pancreatic acinar cells (PACs) in dogs. However, in pigs no information is available whether IF is either predominantly synthesized by GPCs or PACs. Experimentally-induced exocrine pancreatic insufficiency (EPI) could help to rule out that IF is mainly synthesized by PACs. Within cells cobalamin is mainly bound to cobalamin-dependent enzymes, such as methionine synthase and methylmalonyl-CoA mutase. In cells, a malfunction of methionine synthase and methylmalony-CoA mutase due to a lack of cobalamin can lead to increased homocysteine and methylmalonic acid concentrations, respectively. Homocysteine and methylmalonic acid concentrations can be quantified in serum. Therefore, the study aimed to evaluate the effect of experimentally-induced EPI on the intracellular cobalamin status in pigs.

Methods: Age-matched pigs (n=14) were randomly allocated to group A (controls; n=4), group B (experimentally-induced EPI in 7 weeks old pigs; n=5) and group C (experimentally-induced EPI in 16 weeks old pigs; n=5). Group A received a sham laparotomy, whereas group B and C underwent surgical ligation of the pancreatic duct and received no pancreatic enzyme replacement during the trial. All pigs consumed the same diet throughout the study period. Serum samples were obtained from all pigs at 9, 15, 21, and 26 weeks of age. Serum homocysteine, methylmalonic acid as well as cobalamin concentrations were measured at all time points using a homocysteine enzyme-cycling assay, high-performance-liquid-chromatography and electro-chemiluminescence immunoassay, respectively. The effect of EPI on serum cobalamin, homocysteine and methylmalonic acid concentrations was evaluated by using a MANOVA model.

Results: Serum cobalamin (p=0.006) and methylmalonic acid (p=0.004) concentrations but not serum homocysteine (p>0.05) concentrations were significantly different among the groups. Concentrations of serum cobalamin and methylmalonic acid in Group B (mean ±SD: 39.2 ±2.4 pmol/L and 27,630 ±13,375 nmol/L, respectively) were significantly lower and higher for all timepoints, respectively, when compared to group A (74.2 ±10.1 pmol/L, p=0.008 and 644 ±333 nmol/L, p=0.016, respectively) and group C (67.1 ±16.1 pmol/L, p=0.004 and 3,221 ±3,638 nmol/L, p=0.014, respectively). In contrast, no differences were observed between group A and group C for both serum cobalamin and methylmalonic acid concentrations for all timepoints (for both p>0.05).

Conclusion: EPI in 7 weeks old pigs did affect the availability of systemic and intracellular cobalamin, which may suggest that IF is mainly synthesized by PACs in pigs. Further investigations are warranted to evaluate the different response in pigs where EPI was experimentally-induced at 7 and 16 weeks of age. The results of this study demonstrate that experimentally-induced EPI affects intracellular methylmalonyl-CoA mutase more than methionine synthase in pigs. The difference in the response to both cobalamin-dependent enzymes could be that methionine synthase has more than one co-factor.

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43.

Dietary changes in pig nutritional studies shape the structural and functional composition of the pig's fecal microbiome - from days to weeks

Veränderungen in der Ration beim Schwein führen zu einer strukturellen und funktionellen Veränderung des fäkalen Mikrobioms - von Tagen zu Wochen

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The diet composition is one of the major environmental factors shaping composition and activity of the intestinal microbiome. Nowadays, modulation of the type and content of protein and carbohydrates together with food supplements are the most promising strategies to promote gut homeostasis and a balanced intestinal microbiome. Nevertheless, research into the dimension/extent and the duration of microbial adaptation processes is still required, with special focus to be directed to the purpose of evaluating the impact for all nutritional studies where the effects of experimental diets are tested. In this study, amplicon sequencing together with a metaproteomic approach were used to determine the length of the adaptation period for the microbiome to restore its structural balance after the change from a basal diet to an experimental diet.

Methods: Twelve pigs were randomly grouped and fed four different diets varying in the level of supplemented CaP (low (4.4 ± 0.14 g Ca/kg; 4.15 ± 0.07 g P/kg) or high (8.3 ± 0.00 g Ca/kg; 7.45 ± 0.07 g P/kg; supplemented with monocalcium phosphate)) and protein source (peas vs. soybean meal) as described in Heyer et al. (2016) [1]. Fecal samples from three animals per diet were collected at seven time points in the first four weeks after the dietary changes from basal to experimental diets. DNA was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedical). Illumina sequencing of the 16S rDNA (V1-V2 regions) [2] was used to characterize the overall bacterial diversity. Proteins were extracted and further purified by a short 1D gel electrophoresis step. Peptides were created by an in-gel based trypsin digestion and measured by liquid chromatography coupled to mass spectrometry (LC-ESI-MS/MS, Thermo Scientific Q Exactive HF system). The MS/MS data were analyzed through a two-step database search strategy, using both Proteome Discoverer and MaxQuant software for a qualitative and quantitative metaproteome characterization [3].

Results: DNA-and protein-based data of the pig´s fecal microbiome showed significant shifts ($P<0.05$) in its structural composition over the whole experiment, regardless of the diets fed. Fecal microbiota composition prior to the feeding of the experimental diets was different ($P<0.05$) from the community structure assessed after the experimental diets were fed and an adaptation was reached. Several bacterial families changed their abundance. For example, the relative abundance of sequences affiliated to Lactobacillaceae decreased within time, whereas sequence reads belonging to Clostridiaceae and Prevotellaceae increased. Statistical analyses of sequencing and protein data showed the dynamics after the dietary change. A separate sample clustering indicates a step-wise adaptation of the fecal microbiome. The samples are clustered according to the characteristics into start phase (zero), the metabolic adaptation period (MA) and a new stable community (EQ) which is formed after three to four weeks of the experimental trial started. A significant separation according to the level of supplemented CaP occurred only in week four (EQ) after dietary change (*P*-values: 0.710 (zero), 0.033 (MA), 0.001 (EQ)). The identification of about 9500 bacterial proteins in total allows a deep insights into shifts of the metabolic capacity along the time. A core metaproteome of 4380 proteins was identified in samples of all time points. The remaining part of the dataset describes important differences imputable to gain/loss of function as well as a diverse composition of the bacterial community involved in conserved functions. Abundance of methylmalonyl-CoA mutase, acetyl-CoA carboxylase and other proteins indicative of propionate production decrease over the time and is counterbalanced by a progressive increase in the abundance of proteins related to acetate and formate biosynthesis. The butyrate production is mainly predicted by proteins affiliated to the family Ruminococcaceae and Veillonellaceae regardless the time. For acetate production, the progressive decrease of proteins belonging to Veillonellaceae is balanced by the increased abundance of NAD-dependent aldehyde dehydrogenase related to Streptoccoccaceae.

Conclusion: We report insights on the temporal changes of the gut microbiota after a dietary change. The results showed that a stable microbiome stimulated by the dietary treatment is first detectable in three to four weeks after the dietary change. This should be considered for an adequate adaptation period in future pig nutritional studies.

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44.

Effect of supplementing broiler diets with dry whey powder and whey protein concentrate on the productive performance, digestibility of nutrients and cecal microbiota composition

Einflüsse von Molkepulver und eines Proteinkonzentrates aus Molke auf die Produktionsleistung, Nährstoffverdaulichkeit und die mikrobielle Gemeinschaft des Caecum im Broiler

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Modern intensive broilers production systems have a main purpose to promote a high growth rate, feed efficiency and optimal health status of animals [1]. These required conditions can be achieved by using different dietary interventions and by promoting a balanced gut microbiota population. Alternative feed ingredients for broiler diets such as dry whey powder (DWP) and whey protein concentrate (WPC) are expected to improve productive performance and cecal microbiota composition. This work aims to evaluate the effects of supplementing diets with DWP and WPC on productive performance, ileal digestibility of nutrients and cecal microbiota composition.

Methods: One-day-old chicks were distributed into three corn-soybean based diets: control diet (basal diet with no supplementation of DWP or WPC), 60-DWP diet (60 g/kg of DWP) and 80-WPC diet (80 g/kg of WPC). Diets were formulated based on ideal protein concept. External L-lysine and DL-methionine were added in order to meet specific broilers' requirements. A productive performance study was conducted during 42 days. At day 21 digesta samples of the lower ileum were collected, pooled (n=15 per treatment, nine pen per treatment) and lyophilized. The coefficient of apparent ileal digestibility (CIAD) of dry matter (DM), crude protein (CP), calcium (Ca) and phosphorus (P) were estimated using Cr_2O_3 as indigestible external marker. Cecal digesta samples were collected at day 42 from individual birds (n=5 per treatment, five pen per treatment). Total nucleic acids were extracted with a commercial kit and then subjected to Illumina amplicon sequencing. Phylogenetic analysis of the 16S rRNA gene sequences was assessed using Mothur pipeline [2]. Pens were considered replicates for statistical analysis.

Results: An increase on the CIAD of Ca was observed in animals fed with 60-DWP compared to control diet (p=0.041). In regards to CIAD of P, chickens fed with 60-DWP and 80-WPC showed an increment compared to control diet (p<0.001 and p=0.002, respectively). At the end of the starter period, animals fed with 80-WPC revealed higher daily weight gain (DWG), body weight and feed intake (FI) when compared to the control diet (p<0.001) and 60-DWP (p<0.001). For the entire period of feeding, animals fed with 60-DWP or 80-WPC showed higher DWG (p=0.006 and p<0.001, respectively) and FI (p<0.001 and p=0.001, respectively) than control diet. A decrease on feed conversion ratio was observed by feeding with 60-DWP compared to control diet (p= 0.048) while non-differences were found between 80-WPC and control diet.
Statistical differences on the composition of the cecal bacterial community were observed between experimental diets (R=0.476; p=0.001) and a subset of 6 OTUs summarize the overall differences. These OTUs are associated to *Faecalibacterium* spp. (OTU 1), *Bacteroides* spp. (OTU 2), unclassified Porphyromonadaceae (OTU 3), *Bacteroides fragilis* (OTU 5), *Helicobacter pullorum* (OTU 7) and *Escherichia coli/Shigella flexneri* (OTU 9). In comparison to the control diet, animals fed with 60-DWP and 80-WPC diets were colonized in higher abundance with *Bacteroides fragilis*, *Bacteroides* spp., *Escherichia coli/Shigella flexneri* and *Megamonas furniformis*, while *Helicobacter pullorum* was present in lower abundance. *Lactobacillus salivarus* consistently increased in chickens with better FCR that were fed with 60-DWP.

Conclusions: The supplementation of DWP and WPC in broilers diets improve animal growth, feed intake and mineral digestibility. The decrease in abundance of OTU 7, in animals fed with 60-DWP and 80-WPC diets, improve animal health status and reduce the potential foodborne pathogen contamination risk. This study shows an association between performance results, obtained by feeding with DWP and WPC, and cecal microbiota.

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45.

Caecal microbial community response to prebiotic and probiotic supplementation in laying hens diets

Veränderung der mikrobiellen Gemeinschaft des Caecums in Legehennen durch Probiotika und Prebiotika

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The caeca of laying hens harbour a complex and dynamic microbial community that has numerous functions. One of the most important function is to protect the host from intestinal disorders. Prebiotics and probiotics are safe additives that improve animal health and performance. To assess the effect of supplementing corn-soybean diets of laying hens with dry whey powder (DWP) as a prebiotic, *Pediococcus acidilactici* as a probiotic (PA) and the combination of both (DWP-PA) as synbiotic, taxonomical and functional analysis were used to explore microbial variations.

Methods: A total of 300 laying hens with 57 weeks of age, were randomly allocated to floor pens for 70 days. Pens were assigned to four experimental diets with five pens per treatment. The treatments included a control diet, DWP diet (60 g/kg of DWP), PA diet (20 g/kg of PA), and a DWP-PA diet (a mixture of 60 g/kg of DWP and 20 g/kg of PA). Caecal contents were taken from 12 individual hens to proceed with DNA extractions and Illumina amplicon sequencing procedure, targeting the 16S rRNA V1-2 region [1]. Phylogenetic analysis of the 16S rRNA gene sequences was assessed using Mothur pipeline [2]. One way analysis of similarity (ANOSIM) was used to evaluate similarity between different dietary groups, and a p-value lower than 0.05 was considered to be significant different. Illumina amplicon sequencing data were analyzed using statistic software PRIMER6. Metagenomic DNA from 4 individual samples, corresponding to each treatment, was sequenced using Illumina HiSeq2500 platform. The analyses of the metadata were performed through the EBI Metagenomics service pipeline that includes a quality control step, a taxonomic analysis step based on 16S rDNA sequences and a functional analysis of predicted protein coding sequences [3].

Results: A statistical difference was observed between the diets ($p < 0.05$) at operational taxonomy unit. Bacteroidaceae was the most abundant family across all treatments (>15%). Ruminococcaceae was detected in similar abundance in all treatments (12%). The most abundant families in the control and synbiotic diet, with relative abundances between 5 and 19%, were Bacteroidaceae, Prevotellaceae and Porphyromonadaceae. Coriobacteraceae and Lachnospiraceae were detected in higher abundance in samples belonging to synbiotic diet (14% and 8%, respectively), when compared to the other dietary treatments. Samples from PA diet showed lower abundance of Lactobacillaceae (2%). The addition of prebiotic to the diet promotes the presence of genera *Olsenella*, which is known to be a fermenter of carbohydrates and to produce predominantly lactic acid [4]. *Lactobacillus crispatus* tended to be detected when there is addition of DWP to the diet. Around 30% of the reads obtained with metagenomics analysis could not be taxonomically assigned. All metagenomes shared 1204 functions. In each individual metagenome were detected unique functions that were not shared with the others (Control=57; DWP=195; PA=88; DWP-PA=35). Specific genes encoding for functions related to calcium mediated signalling and fatty acid synthase activity were only detected in the prebiotic treatment. In the synbiotic treatment were found alpha amylase and chitinase activities, while the probiotic treatment exhibited genes for the regulation of phosphatase activities.

Conclusions: The dietary treatments tested in this experiment have an influence in the caecal microbiota. Despite the fluctuations observed a core of functions was detected in all four samples analyzed with metagenomics. Specific functions, encoded by the microbial community, were particular for each treatment.

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46.

In situ disappearance kinetics of phosphorus and macronutrients and rumen microbial abundance in cows fed concentrates treated with lactic acid, with or without inorganic phosphorus supplementation

In situ Abbaubarkeit von Phosphor und Makronährstoffen und Abundanz von Pansenmikroben bei Kühen bei Fütterung von mit Milchsäure behandeltem Kraftutter, mit oder ohne Supplementierung von anorganischem Phosphor

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Increasing ruminal degradation of organic phosphorus (P), stored as phytate, in cereal grains may reduce the needs for inorganic P supplementation and minimizes P excretion that pollutes the environment. Grain treatment with lactic acid (LA) was shown to speed up ruminal P degradation at the presence of inorganic P supplementation (1). However, not only the host, ruminal microbes also have specific P requirements for growth and activity. This study investigated whether such an ability of LA treatment to increase P availability and modulate microbiota in the rumen can be maintained even without a provision of readily available inorganic P.

Methods: Six rumen-fistulated non-lactating Holstein cows were assigned to a double 3 × 3 Latin square design with 3 experimental periods, each lasted 14 days. Three diets were tested in each period including untreated control diet supplemented with inorganic P (CON+P), and two LA-treated diets one with (LA+P) and another without inorganic P supplementation (LA-P). For LA treatment, the concentrate mixture was steeped with 5% LA for 24 h before feeding as TMR. All diets contained 53% forage and 47% concentrate on dry matter (DM) basis. As analyzed, the CON+P and LA+P diets contained 4.7 g P/kg DM, whereas the LA-P diet contained 4.1 g P/kg DM solely from organic sources. Following an in situ method done on d 8-10 in the first and last experimental periods, the untreated and both LA-treated concentrates were incubated for 24 h in the rumen of cows fed diets based on corresponding concentrate treatment. The nutrient disappearance of the concentrate was determined at 0, 2, 4, 8, 12, and 24 h of incubation (n=4 per diet per time) to estimate in situ degradation coefficients and effective degradability (ED, %). The level of P in free ruminal liquid (FRL, mg/L) and digesta at 0 and 4 h (g/kg fresh matter) after morning feeding on d13 in all periods (n=6 per diet per time) was analyzed using a vanadomolybdate-based method. Abundances of target microbes in FRL and solid digesta collected at 2 h after morning feeding on d14 in all periods (n=6 per diet) were determined using qPCR. Data were subjected to analysis of variance using the MIXED procedure of SAS. The fixed effects included diet, square and experimental period within square.

Results: Independent of inorganic P supplementation, LA treatment promoted the ED of crude ash ($P<0.05$) and P ($P=0.06$) compared to the untreated control, whereas the ED of OM and CP was unaffected. The differences in crude ash and P disappearance between diets were most pronounced during the first 4 h of incubation as indicated by the higher rapidly degradable fraction (intercept) in both LA diets compared to CON+P ($P<0.05$). All diets reached similar plateaus of the ash and P disappearance after 12 h of incubation. The P supplementation decreased the lag time of starch disappearance ($P<0.05$) and increased the fraction of rapidly degradable starch (LA+P vs. LA-P, $P=0.05$). The P levels in FRL and in digesta were lowest in LA-P at 4 h after feeding, but before feeding the diet effect was found only in digesta. Total bacterial number was unaffected but the genus *Prevotella* thrived on both LA-treated diets in both rumen fractions, whereas the opposite was found for *Butyrivibrio fibrisolvens* and *Selenomonas ruminantium* in solid digesta ($P\leq0.05$).

Conclusions: Regardless of P supplementation, treating the concentrate with 5% LA promoted ruminal disappearance of P and possibly other minerals comprising the ash and caused bacterial shifts, without an effect on the degradation of the macronutrients and total bacterial abundance. The findings suggest that LA treatment can enhance the solubility of organic P at early hours, thereby increasing the availability of P to ruminal microbes. The P levels in the rumen samples were more related to the dietary P supply. Also, a contribution of salivary P to the level and availability of total P in the rumen has to be considered.

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47.

Postruminal digestion of starch infused into the abomasum of heifers with or without exogenous α-amylase administration

Postruminale Verdauung abomasal infundierter Stärke mit und ohne exogener Zulage von α-Amylase bei Färsen

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Increasing starch flow to the small intestine is seen as an approach to improve glucose and energy supply to high yielding dairy cows. The hypothesis of this study was that capacity of small intestinal starch digestion is limited by endogenous amylase activity in the small intestine and might therefore be improved by exogenous amylase administration.

Methods: Four rumen-fistulated heifers (~565 kg bodyweight) were assigned to a 2×2 Latin square trial with two experimental periods lasting 23 d each with 10 d of diet adaption followed by 13 d of abomasal infusion (10 h/d) and sample collection. During the first 3 d of each infusion period an isotonic saline solution was infused (1 L/h) for measurement of basal values, followed by a 10-d infusion of 880 g/d corn starch (suspension of 1 kg/10 L water) with or without exogenous α-amylase solution (2%; 50 mL/h). The heifers were fed 5.5 kg/d of a diet 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 DM, starch, total N, purine bases and short-chain fatty acids (SCFA). Microbial N excretion was estimated from purine bases assuming a purine N: microbial N ratio of 0.116 (1). Starch disappearance in the small intestine was estimated from additional microbial protein synthesis in the hindgut due to starch infusion assuming an efficiency of 1 g microbial N/100 g starch (2). Data were analysed using the MIXED procedure of SAS in a repeated measures model.

Results: Abomasal starch infusion increased faecal DM, total N, microbial N, acetate and butyrate excretions ($P < 0.05$). The molar percentage of butyrate was increased in expense of acetate and propionate ($P < 0.05$). Propionate excretion was not affected by starch infusion ($P > 0.05$). Apparent disappearance of starch was around 99% in the total tract and estimated to be around 85% in the small intestine. None of these parameters were affected by amylase treatment ($P > 0.05$).

Faecal Parameters[1]	Unit	Basal Mean	Starch Δ	Basal Mean	Starch + Amylase Δ	SEM	Amylase effect on Δ P value
DM	kg/d	1.56	+ 0.16*	1.54	+ 0.20*	0.02	0.24
Starch	g/d	1.6	+ 3.2	0.3	+ 3.5	2.0	0.90
Total N	g/d	34.6	+ 7.4*	34.5	+ 6.8*	1.0	0.46
Microbial N	g/d	8.8	+ 1.1*	8.9	+ 1.2*	0.4	0.81
Acetate	g/d	12.6	+ 6.7*	15.0	+ 6.4*	2.4	0.93
Propionate	g/d	2.6	- 0.2	3.1	- 0.6	0.5	0.60
Butyrate	g/d	1.4	+ 5.0*	1.8	+ 3.7*	1.3	0.37

[1] Basal values (mean of 3 d saline infusion) and LSM and SEM of the differences between saline and starch infusion.

* Significant difference between saline and starch infusion ($P < 0.05$). *P* value and SEM not shown.

Conclusion: Increasing microbial N and SCFA excretions indicated an enhanced carbohydrate fermentation in the hindgut. Missing effects of an exogenous amylase administration were probably due to a sufficient activity of endogenous amylase. Therefore, starch disappearance in the small intestine seemed to be limited by restricted glucose absorption or the activity of other enzymes rather than endogenous amylase activity at this level of starch infusion. Further studies are required to clarify whether endogenous amylase activity is a limiting factor for small intestinal starch digestion at higher levels of starch infusion.

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48.

Effect of different particle sizes of barley on apparent total tract digestibility in horses

Einfluss des Vermahlungsgrades der Gerste auf die scheinbare Verdaulichkeit beim Pferd

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Introduction: It is common practice that grains, like barley, are added in diets for horses to enhance their energy status and hence cover the animal's requirement. Barley has the disadvantage of a poor prececal starch digestibility, which can be improved by grain processing techniques like grinding. The aim of this study was to determine the effect of two different levels of mechanical processed barley on the apparent total tract digestibility (ATTD) and the concentration of microbial metabolites in feces when fed to horses.

Methods: In a 3x3 Latin square design twelve mature Warmblood horses (573±72 kg) were assigned to three treatments, whereat: T1 - basal diet (hay + straw), T2 - basal diet + crushed barley, T3 - basal diet + ground barley. Crushed barley was obtained by grinding with roller mill resulting in about 2.2 mm particle size, whereas ground barley was obtained after grinding with hammer mill, resulting in about 0.9 mm particle size on average. Two horses per treatment were fed according to maintenance requirements (1 kg barley/ day), and two horses according to their exercise activity, which was classified (1) as easy work (2 kg barley/ day). Barley supplementation varied from 0.1 % to 0.4 % of body weight. The basal diet:barley ratio was 84:16. The digestibility trial was divided in three replicated periods with ten days of adaption and five days of collection period for each replicate. The feed and feces samples were analyzed for dry matter (DM), crude protein (CP), ether extract (CL), crude fiber (CF), NDF (neutral detergent fiber), ash (CA), starch, sugar and acid insoluble ash (as indigestible marker). Additionally the volatile fatty acids content (SCFA) in faeces was analyzed. The results were evaluated with the statistic analysis system SAS using the mixed linear procedure considering covariances. Statistically significant differences were considered for P-Value <0.05.

Table 1. Digestibility of diets

Nutrient	Diet			SEM	P-Value
	T1	T2	T3		
	Digestibility [%]				
DM	92.3	92.9	93.3	0.3	0.20
CP	35.2	45.3	47.9	3.6	0.04
CL	53.6	58.5	59.8	1.9	0.22
CF	59.5	60.4	61.7	1.7	0.76
NDF	58.7	60.4	61.2	1.8	0.70
CA	34.7	38.8	38.9	1.6	0.15
CSt	100	100	100	n.a.	n.a.
CSu	100	100	100	n.a.	n.a.
	mmol/kg DM				
SCFA	10372	10861	8775	605	0.38

Results: The results showed a higher crude protein digestibility for the barley diets compared to the basal diet ($P<0.05$). The grinding level of barley did not alter the ATTD of nutrients as well as the content of volatile fatty acids in faeces. The ATTD of fat correlated negatively with the amount of acetate as well as with the sum of volatile fatty acids ($r^2=0.37$ and $r^2= 0.39$; $P<0.05$). Furthermore the crude protein digestibility and the total volatile fatty acid content are negatively related ($r^2=0.29$; $P<0.1$).

Conclusion: When choosing mechanical grain treatment, there is no need to generate smaller particle sizes, as long as the content of barley in the ration is low in relation to the basal diet.

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49.

Chewing patterns in horses during the intake of various quantities of two pelleted mixed feeds differing in their physical characteristics only

Kauparameter beim Pferd während der Aufnahme verschiedener Mengen von zwei pelletierten Mischfuttermitteln welche sich nur in ihren physikalischen Eigenschaften unterschieden

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It has been shown that horses ingest pelleted mixed feed (PF) faster than crushed oat grains or muesli feed (1). This rapid ingestion needs to be evaluated critically because of reduced tooth wear, elevated risk for oropharyngeal obstruction and accelerated starch incorporation at least if the PF in question is a starchy one. We hypothesized that i) adjusted physical properties such as elevated size and hardness may counteract these detrimental effects, and ii) there might be interactions between physical properties and the meal size. Aim was to compare feed intake patterns after feeding various quantities of PF originated from the same batch of feed but equipped with different physical characteristics.

Methods: Six warmblood-type mares (age 8.5±3.1 years; bwt 519±36.3 kg; BCS 5.2±0.42/9) were individually housed in box stalls with straw as bedding and received metabolizable energy (ME) according to the maintenance level (0.52 MJ/kg $bwt^{0.75}$ d^{-1}). Two PF´s were produced from the same batch of feed mixture: PF1: ø 5mm, length 21.9±4.97 mm, hardness degree [HD, Amandus Kahl test-spring] 6.9±3.7 kg, water holding capacity [WHC, 2] 1 min 1.39±0.12, 5 min 1.54±0.02, wet sieve fraction [WSF] < 0.2 mm: 31.3% , >1 mm 33.1%; PF2 edge length 15.6±0.14 x 15.6±0.08 mm, length 54.4±9.59 mm, DH 43±9.2 kg, WHC 1 min 1.55±0.04, 5 min 1.57±0.05, WSF<0.2 mm 31.8% , >1 mm 38.9%. Contents of analyzed proximate nutrients and energy were as follows (per kg dry matter [dm]): 136 g crude protein, 55 g crude ash, 302 g neutral detergent fiber, 165 g acid detergent fiber, 45 g acid detergent lignin, 11.1 MJ ME). The horses got the two pellets in 3 different quantities (1, 1.5, 2.0 kg) once per day according to a blocked cross over design with period length of 8 d. Hay covered the remaining energy need. On d7 and d8, feed intake patterns for PF 1 and 2 were measured by a modified halter (1) after offering 1 kg hay according to the recommended feeding practice. SAS® was used to perform ANOVA with 'pellet characteristic' and 'meal size' as main factors.

Results: During adaption to PF2 an extremely high saliva production was observed. With quantities above 1 kg, PF1 *vs* PF2 tended to be ingested more rapidly (tab). Furthermore, PF1 but not PF2 was eaten faster when the meal size exceeded 1 kg (*vs* 1.5 kg, $P<0.05$). There was no difference ($P>0.05$) between the chewing frequency of PF1 *vs* PF2, but 1 kg of PF1 was chewed at a slower rate than 2 kg of the same feed ($P<0.05$). 1.5 and 2.0 kg of PF2 was chewed more intense (chewing intensity [CI] in chewing cycles per kg dm) than PF1 in which significant differences between both pellets ($P<0.05$) were limited to 1.5 kg only. Contrary to PF2, CI was higher with 1 *vs* 1.5 kg of PF1 ($P<0.05$).

Conclusion: The results of the study suggest that chewing patterns are affected by both the physical characteristics of a pelleted mixed feed and the offered meal size. Under the conditions of the current study, larger-sized pellets with a higher HD seem to intensify the chewing process and decelerate the ingestion time, which may be fortunately for the horses' health. Furthermore, interactions exist between physical properties of the pellets and meal sizes, which however might be of importance for both the feeding practice and the design and meaningfulness of related studies.

item		pelleted mixed feed 1 (PF1)			pelleted mixed feed 2 (PF2)			± pooled s.d.
		1 kg	1.5 kg	2 kg	1 kg	1.5 kg	2 kg	
DFI	[min/kg dm]	9.6^{a}	8.3^{b}	8.8	9.3	9.6^{a}	9.5^{a}	0.77
CF	[sec^{-1}]	1.38^{b}	1.42	1.44^{a}	1.39	1.44	1.42	0.023
CI	[kg dm^{-1}]	794^{a}	711^{b}	755	782	827^{a}	811^{a}	66.2

CF, chewing frequency; CI, chewing intensity; DFI, duration of feed intake; $^{a, b, c}$ indicate different means ($P<0.05$)

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50.

Influences of lactation stage and different energy concentrations in roughage on ruminal pH and rumination activity in pluriparous dairy cows

Einflüsse von Laktationsphase und unterschiedlichen Energiekonzentrationen im Grundfutter auf den ruminalen pH-Wert sowie die Wiederkauaktivität bei mehrkalbigen Milchkühen

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Introduction: Subacute ruminal acidosis (SARA) is a critical metabolic disorder in high yielding dairy cows which is suspected to be associated to production losses and a raise in culling rate. This disease can be induced by high portions of concentrates in rations at expense of roughage, which have to be fed in early lactating cows to meet their increased energy demands. The aim of this study was to examine influences of lactation stage and energy supply on ruminal pH and ruminating behaviour.

Methods: In the current experiment 11 rumen-cannulated German Holstein cows were used from three weeks antepartum until sixteen weeks postpartum. During the prepartal period all cows were fed a dry period diet according to GfE recommendations with 80% roughage and 20% concentrates. After calving cows received experimental rations as partial mixed rations (PMR) containing 60% maize silage and 40% grass silage on dry matter (DM) basis. Different amounts of straw were added to roughage to receive either 6.1 MJ NEL per kg DM in the low energy group (LE) or 6.5 MJ NEL per kg DM in the high energy group (HE). Concentrates were fed similarly to both groups at an amount of 250g per kg energy corrected milk depending on expected milk yield of each cow. For each cow the circadian ruminal pH and ruminating activity was measured; weekly during the transition period and every second week after fourth week postpartum. For measurement of ruminal pH a submersible continuous ruminal pH measurement system (Dascor Inc., Escondido, CA, USA) was placed in the ventral sac of the rumen and pH values were recorded every minute. Rumination activity was obtained by the sensor-based automatic measurement system RumiWatch (ITIN+HOCH, Liestal, Switzerland), which detects jaw movements by a flexible pressure tube in the noseband. Means of daily pH and rumination activity were calculated for each day of measurement. For statistical analyses the MIXED procedure of SAS 9.4 for repeated measurements was used with fixed effects of group, time and the interaction between these factors. The cow within the treatment was considered as random effect.

Results: The results of the measurement of ruminal pH and mean rumination time are presented in Figure 1 and 2. In the prepartal period cows had higher pH values compared to early lactation. Near calving time cows had highest ruminal pH-values and lowest rumination activity. Treatment had no effect on ruminal pH (p=0.653) or on ruminating time (p=0.937), but the lactation stage influenced both ruminal pH (p <0.001) and rumination time (p=0.004).

Conclusion: The outcomes of the present study show that the lactation stage and especially the calving event exert significant effects on ruminal pH and rumination time. Moreover, the higher energy concentration in roughage did not influence the measured variables.

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Fig. 1: Mean ruminal pH (LSMeans ± SEM)

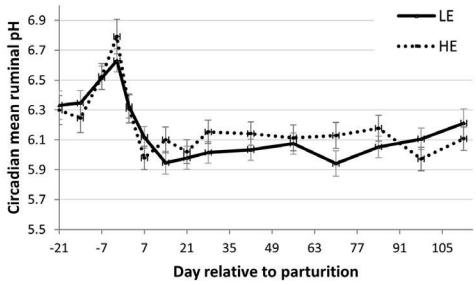
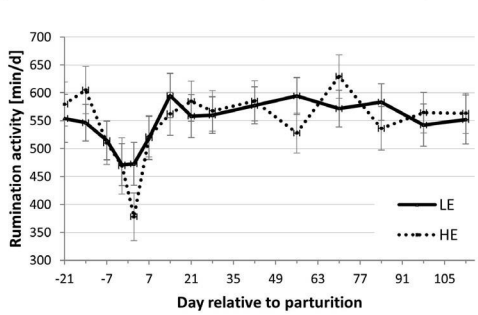


Fig. 2: Mean rumination time (LSMeans ± SEM)



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51.

Use of fat:protein ratio in milk as an indicator of subacute rumen acidosis: A meta-analysis

Der Fett-Eiweißquotient als Indikator für subakute Pansenazidose - eine Metaanalyse

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Early detection is essential for a correct treatment of metabolic diseases like SARA (defined as ruminal pH being <6.15 on average for 24 h and <5.8 for more than 5.25 hours per day (1). At the moment diagnosis largely depends on indicators based on milk composition, like fat content or fat:protein ratio; thresholds between <1.0 and <1.2 are defined as SARA indication for the latter. However, such indicators can be influenced by other factors like stage of lactation. In this meta-analysis we examined milk fat content and fat:protein ratio as predictors of ruminal pH.

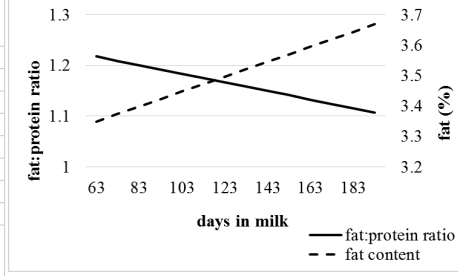
Material and methods: A total of 22 studies containing 91 treatment means were extracted from literature (largely based on (1)). Stage of lactation was 134 ± 34 (mean ± SD) days in milk (DIM) (63 to 192) (min to max). Cows weighed 646 ± 40 kg (563 to 704), dry matter intake was 23.5 ± 2.4 kg/d (17.7 to 28.3) and milk yield was 32.5 ± 6.2 kg/d (20.0-45.2) (4% FCM). Milk fat content was 3.43 ± 0.36 % (2.4 to 4.2), whereas fat:protein ratio was 1.12 ± 0.14 (0.69 to 1.43). The daily average of ventral ruminal pH was 6.0 ± 0.2 (5.6 to 6.4). Data was analysed applying a mixed model (fixed factors: ruminal pH, DIM; random factor: study).

Results: Ruminal pH was significantly and positively associated with fat:protein ratio but not with milk fat content (Tab. 1). For the latter, DIM had a more significant effect than ruminal pH. With increasing DIM estimated fat:protein ratio for a constant pH of 6.15 was slightly decreasing from 1.2 (63 DIM) to 1.1 (192 DIM) (Fig. 1).

Tab. 1: Effects of ruminal pH and DIM on milk fat:protein ratio and fat content

	Regression coefficient	p-value
Milk fat:protein ratio		
Intercept	-0.837	0.0475
pH	0.3428	
DIM	-0.00085	0.0261
Milk fat content		
Intercept	-48.6783	0.0977
pH	16.6928	0.0886
DIM	-0.002456	0.0210
pH^2	-1.3428	0.0999

Fig. 1: Estimated fat:protein ratio and milk fat content at the threshold of pH=6.15 (24 h average)



Conclusion: The meta-analysis indicates that fat:protein ratio is a more meaningful and reliable indicator for ruminal pH than milk fat content. However, some decrease of the fat:protein ratio separating critical and uncritical 24 h pH was present (from the level of 1.2 to 1.1 at later stages of lactation). While pH and DIM were included in our model, further factors influencing milk fat content like e.g. dietary polyunsaturated fatty acids also need to be considered in the future, besides extending observations to the first 60 days of lactation.

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52.

The presence and localization of apelin in the sheep abomasum: impact of diets characterized by different chemical composition

Die Präsenz und Lokalisation von Apelin im Abomasum des Schafes: Auswirkung von Diäten mit unterschiedlicher chemischer Zusammensetzung

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Question: The apelinergic system is a complex system including the apelin peptide (AP), initially isolated in 1998 from bovine stomach homogenates and its receptor, named APJ receptor. The apelin peptide is extensively expressed in several human and laboratory animal organs (heart, lung, brain, mammary gland). The different roles hypothesized for apelin include also the control of blood pressure and the stimulation of drinking behavior in rats. Recently its expression was also evidenced in the basal glandular portion of the stomach in laboratory animals, making it possible to hypothesize its intervention in the control of acid secretion[1,2]. No data concerning its presence and distribution in the abomasum of the sheep are available at the moment. So, we decided to test the presence and distribution of apelin in the abomasum of the sheep and the possible existence of a variability as a consequence of the different chemical composition of the diet.

Methods: The experiment was conducted using 50 "Comisana" sheep fed on Apennine semi-natural pasture for two experimental periods: in the first period the animals were fed for 45 days on pasture at the height of its flowering and this was the first group named group A; in the second period the animals, homogeneous for age, body condition score and milk yield at previous lactation, were fed on the same pasture until it was completely dry while they were equally allocated into two groups (B and C). The group B received a diet supplement of cereals daily (400 g/d/each of maize grain). At the end of each experimental period, the animals were regularly slaughtered at the local abattoir, the abomasum specimens were immediately removed and some of them processed for routine tissue-embedding preparation. They were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. The immunohistochemical reaction was visualized on 5 µm serial sections, using a primary rabbit polyclonal antibody (anti-AP), a secondary biotinylated goat anti-IgG antibody, the avidin-biotin-complex and DAB as chromogen.

Results: The immunohistochemical study showed a peculiar immunoreaction for AP in the abomasums of the animals examined. In particular, an immunopositive reaction for AP was evident in the cells of the basal third of the tubular glands and they were mainly of the closed type, with an oval or round shape and contained many perinuclear granules. The immunopositive reactions did not evidence any difference in either the localization or in the number of the positive cells, among the different experimental groups. Immunopositivity for AP was not observed in any other histological structure or in the sections utilized as negative controls.

Conclusions: These results allow us to conclude that AP is present in the glandular tissue of the abomasums of the sheep, as observed in laboratory animals, with a peculiar cytoplasmatic localization. We hypothesize that AP is involved in the control of gastric secretion, probably via APJ receptor. The AP expression in the stomach does not seem to be influenced by the different chemical composition of the diets.

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53.

The presence and localization of Cannabinoid receptors Type-1 and Type-2 in the swine ileocecal valve: the effects of different physical forms of diet on their expression

Vorkommen und Verteilung von Typ-1- und Typ-2 Cannabinoid-Rezeptoren in der Ileozäkalklappe Schweine bei einer unterschiedlichen physikalischen Struktur des Mischfutters

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Question: The endocannabinoid system is a complex system consisting of endogenous molecules named endo-cannabinoids and cannabinoid receptors. There are two types of cannabinoid receptors, type 1 receptor or CB1 and type 2 receptor or CB2. The CB1 is expressed mainly in the brain but also in some peripheral organs, while the CB2 is expressed mainly in the immune system and in hematopoietic cells even if recently its presence has been evidenced also in some peripheral organs. In this investigation, we considered the pig, an omnivore animal, whose digestive tract displays several analogies with human1. In particular we investigated the effects of different physical forms of the diet on the ileocecal valve (IV) or Bauhin valve, considering the expression of cannabinoid receptors as an indicator for the functional activity of this anatomical structure.
Methods: The experiment involved a total of 32 growing pigs fed ad libitum for 4 weeks with one of the four experimental diets. The four diets were identical for chemical and botanical composition but differed for grinding intensity and compaction: FP - Finely ground pelleted diet (dMEAN, 0.46 mm); CM - Coarsely ground meal diet (dMEAN, 0.88 mm); CP - Coarsely ground pelleted diet (dMEAN, 0.84 mm); CE - Coarsely ground extruded (dMEAN, 0.66 mm) diet. At the end of the experiment, all animals were euthanized and the IV immediately removed and fixed in buffered formaldehyde (2.5% v/v) for 24 h at room temperature. Samples were automatically embedded in paraffin, following routine tissue preparation procedures. On 20 out of 32 samples, the immunohistochemical reactions were visualized on 5 µm serial sections, utilizing goat polyclonal anti-CB1 and rabbit polyclonal anti-CB2 antibodies, the avidin-biotin-complex and DAB as chromogen. Sections in which the primary antibodies were omitted, were used as control for unspecific staining.
Results: The immunohistochemical study showed a peculiar immunoreaction for CB1 and CB2 in the IV of the animals examined. In particular, pigs fed with the CE diet displayed CB1 immunopositive reaction particularly evident in some cells of the epithelial layer, while no other histological structure was involved in the immunoreaction. In all other groups of animals, CB1 immuno-positivity was no longer present in the cytoplasm of epithelial cells. Regarding the CB2 receptor, the immunopositivity was evident in the cytoplasm of neurons, localized in the submucosal and muscular layers and all the groups of animals examined. Immuno-positivity for CB1 and CB2 was not observed in any other histological structure or in the sections utilized as negative controls.
Conclusions: These results allowed us to conclude that CB1 and CB2 receptors are present in the swine IV with a peculiar cytoplasmatic localization that partially reflects the physical forms of the diet. These results confirm the hypothesis that diets determine in the organs, structural and functional adaptations connected with the physical form of the diet2. We highlighted that these adaptations are detectable by different expression of molecules such as receptors for cannabinoids.

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54.

Effects of exocrine pancreatic insufficiency on diversity of the ileal microbiota - studies in juvenile pancreatic duct ligated pigs

Einfluss der exokrinen Pankreasinsuffizienz auf das ileale Mikrobiom - Studien in pankreasgangligierten Schweinen

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Exocrine pancreatic insufficiency (EPI) is characterised by maldigestion and malabsorption due to lack of pancreatic digestive enzymes. In EPI small intestinal bacterial overgrowth (SIBO) is often observed (1). In former studies using the model of the pancreatic duct ligated (PL) pig marked effects on indirect markers for intestinal microbiota (higher lactate and LPS-contents in ileal digesta) were seen (2) but no significant changes were found using traditional cultural techniques [unpublished data]. Molecular methods like comparative 16S rRNA gene analysis allow a more profound investigation of the intestinal microbiota. The hypothesis of this study was that EPI influences diversity of the ileal microbiota in pigs and that the duration of EPI might affect microbiota as well.

Methods: The pancreatic duct was ligated in female cross-bred pigs aged 7 weeks (n=4) or 16 weeks (n=4); 4 sham operated pigs (in week 7) were used as controls (C). Procedures were conducted in accordance with the German Animal Welfare Act and the European Council Directive of 24 November, 1986 (86/609/EEC). No pancreatic enzymes were given to PL-pigs. Animals were euthanized with 26 weeks and ileal digesta was sampled to measure ileal digestibility of nutrients and short chain fatty acids (SCFA) and to characterise the microbial community by using 16S rRNA gene pyrosequencing. Sequences were processed according to MR DNA's pipeline including denoising, OTUs generation and chimera removal. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against database derived from GreenGenes, RDPII and NCBI and binned together on Genus level.

Results: In the PL-pigs the ileal digestibility of all nutrients was significantly reduced. The SCFA pattern was significantly modified (see table 1) and treatment had a significant effect on the community composition ($p<0.05$, permutational ANOVA on hellinger transformed count data). Richness (number of genera) and Shannon diversity were highest in C and decreased with duration of EPI, but differed not significantly due to high individual variations and low sample sizes (see table 1).

Table 1: Apparent ileal digestibility, SCFA pattern and Shannon diversity index of microbiota in ileal digesta

	Controls	PL 16	PL 7
mean ileal digestibility of crude protein (%)	57.8 ± 5.05^{a}	11.9 ± 12.2^{b}	$- 8.30 \pm 25.5^{b}$
mean ileal digestibility of crude fat (%)	79.9 ± 1.57^{a}	18.1 ± 15.8^{b}	21.9 ± 24.1^{b}
Acetate (mean % of å SCFA)	94.0 ± 4.19^{a}	78.8 ± 9.38^{b}	72.3 ± 6.66^{b}
n-Butyrate (mean % of å SCFA)	1.87 ± 2.68^{a}	16.3 ± 6.33^{b}	20.4 ± 8.84^{b}
median number of genera	73.5 ± 24.0^{c}	58.0 ± 48.7^{c}	45.0 ± 38.5^{c}
median Shannon diversity index	1.48 ± 0.53^{c}	1.30 ± 0.60^{c}	1.25 ± 0.40^{c}

a,b indicate $p<0.05$ ANOVA GLM procedure and Tukey-Kramer test; c,d indicate $p<0.05$ Kruskal-Wallis test

Discussion: Overall community composition did significantly differ between groups. Our data indicate that the duration of EPI might be of relevance for the composition of the microbiota as both richness and diversity trended to change with disease length (due to low sample sizes and high variations those parameters did, however, not reach significance) indicating that the changes in ileal microbiota are not completed after a period of 10 weeks of EPI. Up to now, investigations on changes of the ileal and faecal microbiota in EPI are missing. Animal studies offer he opportunity to gain deeper insights into the small intestinal microbiota and its role for host health since human derived samples are usually restricted to faecal samples only.

Conclusion: Our results indicate that EPI alters the composition of the ileal microbiota. Further investigations are required to check whether enzyme therapy can reduces these effects.

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55.

Growth performance and digestive capacity of mealworms (*Tenebrio molitor* L.) fed with low or high fiber diets

Wachstumsleistung und Verdauungskapazität bei Mehlwürmern (Tenebrio molitor L.) mit faserarmer oder faserreicher Fütterung

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Edible insects are discussed to gain in relevance as a future source of food and/or feed. So they should be considered as food producing animals similar to common livestock species. As knowledge about growth performance as well as the requirements for and the utilization of nutrients by insects is rare, an experimental feeding study on mealworms has been conducted.

Question: As dietary fiber is poorly digestible for monogastric animals, are there any effects of the dietary fiber content on growth performance and nutrient utilization of mealworms, too?

Methods: The study involved a total of 5,000 mealworms (larvae of *Tenebrio molitor*) which were divided into ten groups of 500 animals each. They were randomly assigned to one of two treatments and were fed with isonitrogenic diets different in fiber contents. Diets were based on a commercial feed for weaned piglets without or with an addition of 20 % wheat bran (low fiber versus high fiber) and a contemporary reduction of cereal contents. Contents of NDF (neutral detergent fiber) in feed were 131 g/kg in the low and 182 g/kg in the high fiber diet (based on DM). Counts of animals, body weight, feed consumption, and feces excretion were recorded weekly. After 21 days larvae were frozen. Nutrient contents were determined in animals and feces. Data analysis included a two-sided T-test (SAS 9.4).

Results: Survival rate (45 % in low fiber; 47 % in high fiber group) and growth performance of mealworms were not affected by treatment. Feed consumption was 13 % higher in the high fiber group worsening feed-to-gain efficiency significantly (Table 1). Digestibility of dry matter (DM) and crude protein (CP) was lower in the high fiber groups. Concentration of dry matter and crude protein in the body of mealworms were equal for both diets. Content of lipids (ether extracts) tended to be higher, and content of crude fiber (CF) was significantly higher in mealworms on high fiber diets. The efficiency of transforming feed nutrients (DM, CP) to mealworm body contents (retention efficiency) was significantly higher with the low fiber diet.

Table 1: Effects of fiber content in the diet on growth performance, nutrient contents and nutrient conversion of mealworms *(Tenebrio molitor)* (means±SEM)

Treatment	Low fiber	High fiber
Wheat bran in the diet, g/kg	4.0	23.2
Dietary NDF (neutral detergent fiber), g/kg DM	131	182
Daily weight gain, mg/mealworm	3.83 ± 0.15	3.96 ± 0.15
Daily feed consumption, mg/mealworm	5.46 ^{a} ± 0.22	6.17 ^{b} ± 0.09
Feed-to gain conversion, g/g	1.20 ^{a} ± 0.06	1.33 ^{b} ± 0.07
Nutrient digestibility		
Dry matter, %	62.0 ^{b} ± 2.8	56.0 ^{a} ± 2.8
Crude protein, %	50.8 ^{b} ± 3.7	44.3 ^{a} ± 3.6
Nutrient contents of mealworms		
Dry matter, g/kg	347 ± 12	350 ± 3
Crude protein, g/kg DM	496 ± 14	497 ± 9
Lipids, g/kg DM	329 ± 12	346 ± 13
Crude fiber, g/kg DM	93 ^{a}± 8	119 ^{b}±16
Retention efficiency		
Dry matter, %	0.21 ^{b} ± 0.01	0.20 ^{a} ± 0.01
Crude protein, %	0.59 ^{b} ± 0.02	0.53 ^{a} ± 0.02

Different lowercases mark significantly different treatment between means (P<0.05)

Conclusion: Reduced digestibility coefficients of DM and CP at higher dietary NDF suggest that fiber is poorly digestible to mealworms, similar to monogastric species like pigs and poultry. But, in analogy to poultry, mealworms compensate a lower nutrient digestibility by raising feed intake which worsens feed-to-gain efficiency. Obviously, the digestive capacity of mealworms is similar to monogastric livestock. Therefore, optimal zootechnical performance of mealworms requires highly digestible feed mixtures with low fiber contents.

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56.

Effects of moderate feed restriction and dietary L-arginine supplementation on cellular and humoral parameters of an acute lipopolysaccharide induced innate immune response in cockerels of a dual-purpose breed

Einflüsse moderater Futterrestriktion und diätetischer L-Arginin-Supplementation auf zelluläre und humorale Parameter einer akuten Lipopolysaccharid-induzierten, angeborenen Immunantwort bei Junghähnen einer Zweinutzungsrasse

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L-arginine (Arg) is dietary indispensable for birds and possesses immunomodulatory properties due to its function as precursor of cytotoxic nitric oxides (NO) and cell proliferating polyamines. To maintain its basic functions and generate a protective innate immune response (IIR), the avian immune system requires a sufficient supply of energy and nutrients. However, as a result of its rapidly increasing metabolic activity and cytokine-mediated anorexia, the acute systemic IIR is accompanied by a severe dysregulation of energy and nutrient homeostasis as well as a subsequent mobilisation of endogenous resources. Considering this tense metabolic state as highly susceptible to nutritional stress, the present study examined effects of surplus dietary Arg on cellular and humoral parameters of an acute lipopolysaccharide (LPS)-induced IIR in moderate restrictively fed chickens.

Methods: A total of 32 one-day-old Lohmann Dual cockerels were commercially reared in a floor-range system for three weeks and in single metabolic cages from day 22 onwards. At day 28 birds were randomly assigned to two diets differing in their Arg concentration only (23.2 % CP; control-diet: 1.37 % Arg; Arg supplemented diet (Arg-diet): 2.04 % Arg; n=16 birds/diet). In addition, from day 28 to 50 both groups were subject to an *ad libitum* and restrictive regime of feeding, respectively (n = 8 birds/diet*regime). During the entire study the restrictive regime limited birds' feed intake to 75 % of *ad libitum* consumption. On day 50 four cockerels of each group were intramuscularly injected with 2 mg *E.coli* LPS/kg BW (Sigma-Aldrich) as IIR inducer and 1 ml of 0.9 % saline solution (NaCl; B. Braun) as negative control, respectively. At 0, 1, 3, 5, 7 and 24 hours *post injectionem* (h *p. inj.*) rectal body temperature was recorded and heparinized blood samples were collected from cockerels' wing vein. Blood samples were examined for parameters of the cellular IIR (e.g. absolute and relative leukocyte counts, heterophil/lymphocyte ratio; HLR) via Wright-Giemsa stained blood smears and those of the humoral IIR (e.g. plasma NO, measured as its metabolite nitrite, via Griess assay kit; Cayman Chemical). Statistical analysis was performed as 2 x 2 x 2 x 6 four-factorial ANOVA (diet, regime, injection and sampling) using SAS procedure MIXED ($p \leq 0.05$).

Results: The regime of feeding and dietary Arg concentration did not affect body temperature (41.1±0.1°C), plasma nitrite level (3.0±0.7 µM), differential blood count and HLR (0.5±0.1) in NaCl treated chickens. Therefore, these birds were just graphically pooled in order to ease readability of Fig. 1. In contrast to NaCl, LPS induced an acute systemic inflammation characterised by an initial hypothermia (40.6±0.1°C) with subsequent moderate hyperthermia (41.5±0.1°C), a severe increase in plasma nitrite levels (Fig. 1b) and a marked leukopenia (Fig. 1a) with strong lymphopenia and heterophilia peaking in a HLR of 3.4±0.3 at 7 h *p. inj.* ($p<0.001$). Whereas surplus dietary Arg did not modify the examined parameters in LPS treated birds, the moderate feed restriction caused lower total leukocyte counts (Fig. 1a) and absolute heterophils counts at 5 to 7 h *p. inj.* as well as considerably higher plasma nitrite levels at 3 to 7 h *p. inj.* (Fig. 1b) compared to *ad libitum* feeding ($p<0.01$).

Conclusions: The present study did not reveal any modulatory effects of surplus dietary Arg on cellular and humoral markers of avian IIR. On the contrary, it indicated that cellular IIR components seem to be more susceptible to metabolic stress caused by moderate long-term feed restriction than humoral components. However, based on the high cytotoxicity of NO against pathogens as well as host tissues, a metabolically intensified NO synthesis has to be considered as two-edged sword for the outcome of an acute IIR further. In conclusion, the present study emphasised the strong dependence of functionality and controllability of avian IIR on immune system's supply of energy and nutrients.

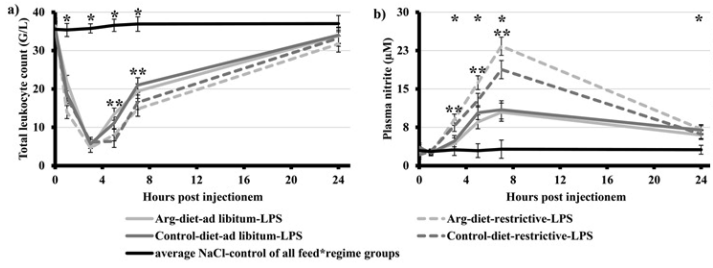

Figure 1. Changes in **a)** total leukocyte count and **b)** plasma nitrite concentration in moderate restrictively fed cockerels supplied with additional dietary Arg during 24 hours post LPS or NaCl injection (LSMeans ± SE; n=4). Values with * differ significantly between the NaCl and LPS treated groups ($p < 0.001$). Values with ** differ significantly between restrictively and *ad libitum* fed LPS-groups ($p < 0.01$).

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57.

L-Arginine requirements for growth and carcass parameters in broilers

L-Arginin-Bedarf für Wachstum und Körperparameters in Masthähnchen

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Environmental impact due to animal production compels the nutritionists to reduce the overall CP of the diet and meet the animal requirements through crystalline amino acids. L-Arginine (Arg) is 5th limiting amino acid in commercial broiler CSBM based diet after valine (1). Comparatively a few studies are available to determine the Arg requirement of broilers. The objective of the present study was to determine the Arg requirement in broilers through performance parameters, breast yield and abdominal fat.

Methods: In total 1440 male chickens (Ross 308) were distributed into 48 pens, and assigned one of the six diets. The basal diet was comprised of corn, wheat and SBM, the feeding program was consisted of a starter (0-14d) grower (14-28d) and finisher (28-35d) periods. The CP and ME contents were 21.9 and 12.7, 20.0 and 12.96, and 18.5% and 13.17 MJ/kg of feed in starter, grower and finisher periods. The Arg:Lys ratios were 0.77, 0.85, 0.95, 1.05, 1.15 and 1.25 in all periods. The Lys levels were 1.29, 1.09 and 1.02% in starter, grower and finisher diets. The performance parameters were calculated for 0-28 days only. As Arg:Lys can be influenced by the higher ambient temperature in finisher period, the performance could not be established from 28-35d due to high mortality because of heat stress during the last week of study. At 35 days, five chickens per pen were killed, exsanguinated and plucked. The abdominal fat and breast meat was weighed and compared, as a percentage of live body weight. Two way (Arg:Lys; location in house) ANOVA was applied and Duncan's multiple range test was used to separate treatment means at the $P < 0.05$ level.

Results: Birds showed a significant ($P<0.05$) response to Arg supplementation as compared to control. Based on regression analysis, the maximum response was achieved beyond Arg:Lys=1.05. The maximum response for BW, F:G was achieved at Arg:Lys=1.15:1.00 for starter period and 1.25:1.00 for grower period. Fat and water contents in animal body are inversely proportional, whereas, water and protein are directly proportional. Therefore, if there is less fat, there is more protein. Arg supplementation linearly increased breast meat and reduced the abdominal fat. The maximum response for breast meat yield achieved at Arg:Lys=1.15:1.00 and for abdominal fat 1.25:1.00 respectively.

Table 1: Performance and carcass parameters

Arg:Lys	BW 14 d	F:G 14 d	BW 28 d	F:G 28 d	Breast yield	Abdominal fat
	g	g/g	g	g/g	%	%
0.77	490^{c}	1.26^{a}	1616^{b}	1.44^{a}	17.70^{c}	2.03^{a}
0.85	528^{b}	1.22^{b}	1690^{ab}	1.38^{b}	18.10^{bc}	1.81^{abc}
0.95	529^{b}	1.21^{b}	1690^{ab}	1.38^{b}	19.10^{ab}	1.89^{ab}
1.05	543^{ab}	1.17^{c}	1710^{a}	1.35^{bc}	18.60^{abc}	1.80^{abc}
1.15	546^{a}	1.16^{c}	1773^{a}	1.34^{c}	19.50^{a}	1.61^{bc}
1.25	553^{a}	1.17^{c}	1778^{a}	1.33^{c}	18.80^{abc}	1.54^{c}

Conclusion: The present study suggested higher Arg:Lys=1.15-1.25:1.00 for maximum performance as compared to the Ross 308 guidelines (2). Breast meat deposition at the expense of fat, suggesting that Arg favours partitioning of energy toward protein deposition at the expense of fat (Arg:Lys=1.15:1.00).

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58.

Impact of different methionine isomers and DL-2-hydroxy-4-methylthiobutyrate (HMTBA) on the quantitative methionine and cysteine metabolism in weaned pigs

Einfluss von verschiedenen Methioninquellen und DL-2-hydroxy-4-methylthiobutyrat (HMTBA) auf den quantitativen Methionin- und Cystein-Stoffwechsel beim Absetzferkel

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Methionine (Met) has several functional roles in the organism. In the intermediary metabolism, Met is transmethylated (TM) to homocysteine, which is either converted to cysteine (Cys) in a process called transsulfuration (TS) or remethylated (RM) back to Met. Several authors suggested increased TS arising from the dietary intake of the Met analogue DL-2-hydroxy-methylthiobutyrate (HMTBA)[1, 2]. However, whole body Met kinetics have not been quantified under HMTBA treatment yet. The objective of this study was to determine to which extent dietary DL-HMTBA, DL-Met or L-Met supplementation to a Met-deficient diet affect whole body Met and Cys kinetics in weaned pigs.

Material and Methods: Forty-five male, weaned German Landrace pigs (8 ± 1.5 kg BW) were randomly allocated to 4 dietary groups: Met-deficient control diet (C, 75% of Met recommendation, n=12; made isonitrogenous by alanine), C supplemented with 0.15% (equimolar basis) L-Met (n=12), DL-Met (n=11) or HMTBA (n=10). The Cys content in the diets did not differ (0.29% of dry matter). Pigs, fitted with catheters, received a primed, continuous intravenous 9 h-infusion of L-[1-^{13}C; methyl-$^{2}H_3$]-Met and [3,3-$^{2}H_2$]-Cys to measure TM and RM rates, TS and protein synthesis (PS) rates as well as Cys flux. Plasma concentrations of the amino acids Met, Cys, cystathionine, glycine and taurine were measured in fasted and fed state by HPLC. Effects of group, state and interaction were analyzed by repeated measures ANOVA using PROC MIXED of SAS.

Results: No differences in body weight (BW) among treatments were observed from age d 28 to 45. However, BW on d 52 of age was higher for L-Met than C pigs ($P < 0.05$). At d 60 of age, the BW of L-Met, DL-Met and HMTBA pigs ($P < 0.05$) were greater compared to C pigs. Whole body Met kinetics in the fed state are shown in Table 1. The fed state Cys flux did not differ between groups. Fed state Met plasma concentrations were lower in C compared to L-Met, DL-Met and HMTBA groups ($P < 0.001$). No differences in fed state plasma Cys, cystathionine and glycine concentrations were observed ($P > 0.05$). Fed state taurine plasma concentration was higher in L-Met compared to C pigs ($P < 0.01$).

Table 1.Fed state whole-body Met kinetics (µmol × kg^{-1} × h^{-1}) in 47 days old piglets fed 4 diets differing in Met level and Met isomers starting at weaning (day 28)

	Diets				
	Control	L-Met	DL-Met	HMTBA	PSEM
TM	23^{a}	49^{b}	44^{b}	44^{b}	2.7
RM	14^{a}	21$^{a, b, c}$	18$^{a, b}$	26^{c}	2.0
TS	9^{a}	28^{b}	26^{b*}	19$^{b*, c}$	2.1
PS	71^{a}	91^{b}	103^{b}	94^{b}	4.2

Least square means (LSM) with different superscript letters within the row are different between groups ($P < 0.05$). LSM within the row assigned with an asterisk tended to be different between groups ($P < 0.1$).

Conclusion: Dietary DL-Met, L-Met and HMTBA supplementation was equally effective in terms of the intermediary Met metabolism. Our results do not support earlier reports[1, 2] that HMTBA supplementation leads to increased levels of TS metabolites compared to the other Met isomers

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59.

Protein quality of chicken diets with complete substitution of soybean meal by insect meal (*Hermetia illucens)* or algae meal (*Spirulina platensis)* and graded fortification of dietary amino acid supply

Beurteilung der Proteinqualität von Broilermischungen mit vollständigem Ersatz von Sojaextraktionsschrot durch Insekten- oder Algenmehl bei gestufter Verbesserung des Aminosäurenangebotes

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The high contribution of extracted soybean meal (SBM) as a protein source in mixed feed for growing chicken is termed as "not sustainable" and requires more alternative protein sources. The larvae of the black soldier fly Hermetia illucens and the blue green algae Spirulina platensis are pointed out as future alternatives and in focus of several research projects. The aim of the current study as part of the multidisciplinary project "sustainability transitions" was the evaluation of protein quality parameters of chicken diets with complete substitution of SBM by partly defatted Hermetia meal (HM) or Spirulina meal (SM) based on extended N balance studies with meat type chickens.

Methods: N-balance studies were conducted with male growing chicken (Ross 308) both during starter period (35 birds, 10-20d) and grower period (35 birds, 25-35d). Following 5d adaptation excreta were collected in two consecutive quantitative collecting periods of 5d each. Five diets were under study. The starter/grower control diet was based on wheat (33/38%), corn (16/19%) and SBM (39/32%) as main ingredients. In experimental diets SBM was completely substituted by SM and HM, respectively. Consequently, final starter/grower diets contained 21/17% SM and 26/22% HM in order to ensure recommended CP levels (22/20%). Diets with both of the alternative proteins were fortified with supplemented amino acids (AA) on a basic level (Lys, Met) according to the control diet and an extended level (Lys, Met, Thr, Arg, Val, Ile, His) according to the currently recommended (1) ideal AA ratio (IAAR). Excreta and feed analyses were in agreement with standard procedures of VDLUFA. N balance data analysis applied the "Goettingen approach" (2) and evaluated the dietary protein quality both by net protein utilization (PNu) and standardized net protein utilization (PNustd) according to (3), making use of a standardized daily N-intake (NI) of 3000mg/BWkg0.67. Statistical analysis (one-way ANOVA, Tukey-test, Games-Howell-test) run by SPSS (Statistics 24).

Results: The results (Table) of control diet exceeded all the other diets regarding the dietary protein quality parameters under study. Extended AA supplementation of both diets with alternative protein sources improved dietary protein quality significantly when PNustd was applied, indicating misleading evaluation of dietary protein quality when the standardization on NI is failed.

Diets	Control +Basic AA	HM +Basic AA	SM +Basic AA	HM +Extended AA	SM +Extended AA
1) PNu [%]	75.8c ± 2.5	65.9a ± 6.4	67.6ab ± 8.5	72.0b ± 3.3	71.8b ± 2.9
1) PNustd [%]	81.2c ± 2.5	62.6a ± 4.6	60.2a ± 5.1	79.8c ± 2.8	75.2b ± 3.7
2) PNu [%]	77.7^{c} ± 4.3	64.8^{a} ± 5.0	68.8^{ab} ± 7.5	69.0^{ab} ± 2.5	70.0^{b} ± 2.4
2) PNu_{std} [%]	84.5^{c} ± 6.0	63.8^{a} ± 4.5	60.9^{a} ± 5.5	72.1^{b} ± 3.2	74.7^{b} ± 3.5

1) = starter period; 2) = grower period; Means (± SD); PNu= net protein utilization; PNu_{std}= standardized net protein utilization (standardized N-intake = 3000mg/$BW_{kg}^{0.67}$); different superscript letters reveal significant differences between diets ($p \leq 0.05$).

Conclusion: Complete replacement of SBM by partly defatted *Hermetia* meal or *Spirulina* powder in chicken diets depressed dietary protein quality, but advanced AA supplementation improved protein quality significantly. This important response became only evident when the well-known effect of N intake on protein utilization was eliminated by standardization of N-intake. Ongoing research will further optimize the dietary AA balance when 100% SBM is substituted by alternative proteins under study.

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60.

Experimental studies on effects of ad libitum feeding and arginine supplementation on performance of sows and piglets

Experimentelle Untersuchungen an laktierenden Sauen zu Effekten einer ad libitum Fütterung und einer Argininzulage auf die Leistung von Sauen und Ferkeln

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Sows' reproductive performance has increased markedly within the last few years. Therefore sows must be supplied appropriately with energy and nutrients for an optimum in milk production and to prevent massive losses of body substance. There are studies in sows that discuss positive effect of arginine on the blood flow. Therefore arginine supplementation might enhance lactation performance [1]. The hypothesis of this study was that sows' feeding management in the form of an ad libitum offer or a combination of ad libitum offer and higher dietary arginine levels might foster piglets' development and prevent too high body substance losses during lactation.

Methods: In experimental period a total of 71 sows in 3 groups (G1-3) were fed by 2 different feeding and dietary regimes. The 24 sows of G1 and G2 were fed a common non pelleted lactation-diet (per kg diet - fresh matter; G1: 12.9 MJ ME, 52.6 g XF, 168 g XP, 10.4 g Arg; G2: 12.9 MJ ME, 54.3 g XF, 166 g XP, 11.0 g Arg). In G1 a manually controlled restricted feeding scheme was followed (once a day in the morning). In G2 the 26 sows were fed ad libitum by using a special feeding dispenser (2 kg feed in the morning manually and the rest via dispenser). In G3 the 21 sows were also fed ad libitum (technique of feeding was identical). This non pelleted lactation-diet was supplemented with 1 % L-Arginine-HCl (per kg diet - fresh matter; G3: 13.2 MJ ME, 49.1 g XF, 187 g XP, 17.7 g Arg). In all groups sows received the lactation-diet from 3 days post partum until weaning. Sows' feed intake (FI) was measured daily over the whole lactation period. On day -7 and 3 days before weaning body weight and backfat thickness of each sow was determined by ultrasound (Lean-Meater®, Renco, Minnesota, USA) by means of a three-point measurement method according to Müller and Polton (2004). Piglets' birth weight was determined within 24 hours post natum and 1 day before weaning). Statistical analysis was performed with SAS for Windows. To compare parameters between groups one way ANOVA was performed ($p < 0.05$).

Results: The data shows that sows' FI under ad libitum feeding conditions was significantly higher than FI of restrictively fed sows (Table 1). Furthermore changes in body weight are remarkable between the different feeding regimes (d-7: G1=281±23.0^{a} kg, G2=292±23.9^{a} kg, G3=279±22.4^{a} kg; d27: G1=230±21.2^{b} kg, G2= 254±25.2^{a} kg; G3=247±21.2^{a} kg). Feed-restricted sows lost significantly more body weight (G1: 51.8±16.5 kg) than sows of the ad libitum groups (G2: 37.6±16.7 kg; G3: 31.3±18.7 kg). Similar results were found concerning the loss of backfat during lactation. Sows of G1 lost significantly more backfat than those of G2 and G3 (4.71±2.16 mm vs 2.96±1.91 mm and 2.62±1.12 mm). Neither loss of body weight nor loss of backfat significantly differed between G2 and G3. Significant differences between all three groups appear in body weights of piglets at time of weaning. Body weight of piglets in G1 (7.41±1.54 kg) was lower than that of piglets of ad libitum fed sows in G2 (8.03±1.77 kg). Sows fed ad libitum arginine supplemented diets weaned the heaviest pigs of all groups (8.32±1.67 kg).

Table 1: Feed intake of sows (FI; as fed; G1-restricted; G2-ad libitum; G3-ad libitum + 1 % L-Argnine-HCl), duration of suckling period (DSP), body weight of piglets at weaning (BWW), litter growth (LG), body weight losses (BWL) and back fat loss (BFL) in sows during the exp. period

Group	sows	piglets	FI	DSP	Ø BWW	LG	BWL	BFL
	n	n	(kg)	(d)	(kg)	(kg)	(kg)	(mm)
1	24	274	4.91^{b} ± 0.34	27.0^{a} ± 2.93	7.41^{c} ± 1.54	66.9 ± 10.7	51.8^{a} ± 16.5	4.71^{a} ± 2.16
2	26	287	7.10^{a} ± 0.74	27.3^{a} ± 1.50	8.03^{b} ± 1.77	74.3 ± 14.8	37.6^{b} ± 16.7	2.96^{b} ± 1.91
3	21	229	6.91^{a} ± 1.02	27.1^{a} ± 1.61	8.32^{a} ± 1.67	73.8 ± 18.1	31.3^{b} ± 18.7	2.62^{b} ± 1.12

$^{a, b, c}$ averages differ significantly within a row ($p < 0.05$)

Conclusion: Ad libitum feeding regime and arginine supplementation seems to have both positive effects on sows' body composition during lactation. Losses in body weight and backfat over lactation were lower than those of restrictedly-fed sows. In addition the supplementation of L-Arginine-HCl in lactation-diets in a concentration of 1 % seem to support the development of piglets.

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61.

Excretion of faecal, urinary urea and urinary non-urea nitrogen by four ruminant species as influenced by dietary nitrogen intake: a meta-analysis

Einfluss der Höhe der Stickstoff-(N)-Aufnahme auf die Ausscheidung von Kot-N sowie von Harnstoff-N und Nicht-Harnstoff-N im Harn bei großen und kleinen Wiederkäuern: eine Meta-Analyse

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The quantification of faecal nitrogen (FN) excretion and of urinary urea-N (UUN) and urinary non-urea-N (UNUN) excretion at varying N contents in ruminant rations is an important tool in assessing endogenous N turnover via the rumino-hepatic cycle. This meta-analysis therefore aims to pursue and investigate the following hypothesis on the basis of an updated, expanded data set: Regardless of the species or category of ruminant (dairy cattle, growing cattle, sheep or goats), both FN and UNUN are unaffected by a variation in N supply and can therefore be seen as obligatory for derivations of N requirements.

Methods: The database was constructed from 50 publications. The breakdown is as follows: 27 publications on dairy cattle, 6 publications on growing cattle, 10 publications on sheep and 7 publications on goats. The crucial selection criterion in each case was that all relevant N fractions in faeces and urine were quantified, rather than calculated or derived. The data were categorised into dairy cattle, growing cattle (bulls and heifers), sheep and goats. Data from 50 publications were considered. Data were analysed using mixed model regression methodology. The N intake (NI, g/day) was treated as the independent variable and considered as fixed effect. Different studies were considered as random effects. The dependent variables were the daily quantities (g/day) of FN, urinary-N (UN), UUN, UNUN and N retention (NRet).

Results: The FN correlated positively with increasing NI in all species, in the order goats (quadratic model), dairy cattle, growing cattle and sheep. Only in sheep this effect was not statistically significant. The UN also correlated positively with increasing NI in all species. For UN, the clearest effect was again shown in goats, in a quadratic model with a slope value of 2.57, followed by growing cattle (quadratic model), dairy cattle and sheep. This effect was significant for all species ($P<0.05$). The UUN also showed a positive correlation with increasing NI for all four species. For UUN, the clearest effect of a variation in NI was shown for sheep, with a slope value of 3.53 in the quadratic model, followed by goats, dairy cattle and growing cattle. All effects were significant ($P<0.05$). For UNUN, no values could be ascertained for goats due to the limited data set. Sheep and growing cattle showed a positive correlation with increasing NI, and dairy cattle a negative correlation. With a slope value of 11.02 in growing cattle, a stronger influence was identified following NI variation. A clear influence can also be seen in sheep, with a slope value of 2.21. For dairy cattle, with a slope value of -1.01, a negative effect was found after increasing the N intake. However, the effect was statistically significant only in growing cattle ($P<0.05$). In the case of NRet, all four species showed positive correlations with increasing NI. Once again, it was in goats that NRet was most dependent on NI variations (slope value = 2.49), followed by growing cattle, sheep and dairy cattle. However, this effect was statistically significant only in growing cattle ($P<0.05$).

Conclusion: On the basis of an updated, expanded data set, this meta-analysis corroborated for all species studied that UN and UUN in particular are clearly dependent on NI. In contrast, FN and UNUN should be seen as obligatory and are therefore influenced only marginally by increasing NI. The results for FN also show clearly that, in most of the trials, two linked variables influence microbial protein synthesis, namely dietary CP and feed digestibility. The hypothesis that FN should be seen as obligatory can be proven in principle. However, in order to obtain even better derivations of quantitative requirements, it might be necessary to separate a variation in N supply from a variation in digestibility of feed and total ration. This was not possible on the basis of the available data.

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62.

Does the amino acid composition of the equine hoof horn correspond with the observed hoof horn quality in horses?

Korrespondiert die Aminosäurezusammensetzung des Hufhorns mit der Hufhornqualität beim Pferd?

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The quality of the hoof is a significant factor of health, market value and suitability of horses for training. However, data are extremely scarce due to parameters of quality depending on composition of the equine hoof. Earlier observations have indicated that fatty acid composition and hoof horn quality (HHQ) could be correlated (1). The hypothesis of the present investigation is that amino acid (AA) composition of the equine horn protein fraction could also be related to quality parameters of the hoof horn as indicated by (2) due to varying protein supply in ponies.

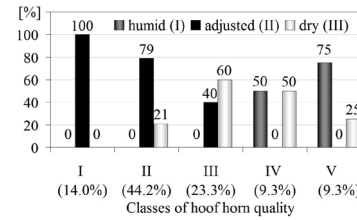
Methods: Hoof samples of totally 39 horses from the field were involved, varying both in sex (16 mares, 23 geldings) and age (4 to 17 years) in 2 periods of sampling (summer 2014 and 2015). 30 horses were from warm-blood type, 9 horses were polo ponies without detailed knowledge about feeding (hey, oat, muesli individually by owners depending on sporting activity). Individual HHQ and hoof horn moisture (HHM) was evaluated based on long-time experience schedule by a professional blacksmith providing five classes of HHQ: (I) excellent, (II) good, (III) medium, (IV) bad, (V) insufficient quality and three classes of HHM: (I) humid, (II) adjusted and (III) dry. Samples were collected from different parts of the hoof and mixed from all four legs and can be defined as mixed sample containing both wall and sole horn fractions. Sample preparation for chemical analyses started with crude cleaning from adherent material, followed from freeze drying and milling (0.5 mm). Analysis of dry matter and nitrogen content (Dumas method) was according to standard procedures of VDLUFA. Samples for AA analysis were prepared according to Commission Regulation (EC) No 152/2009. Amino acids were analysed by ion exchange chromatography (Biochrom®30 AA analyser) to create the specific AA profile of 16 AAs in the hoof protein fraction. Results were statistically evaluated by one-way ANOVA (IBM SPSS Statistics 22) using post-hoc Tukey or Games-Howell test to identify significant differences ($p < 0.05$).

Results: For 58.2 % of horn samples, an excellent and good quality was attested (Figure). The figure shows the relationship between HHQ and HHM without any verifiable coherence between AA profile and HHM. A decrease of HHQ is accompanied by more dry or humid hoofs. The average AA profile of equine hoof protein demonstrates (Table), that in contrast to other tissues, particularly Cys has a much higher concentration due to its importance as component of hoof keratin.

Average AA profile of hoof protein (g AA/16 g N) from period 2

Lys	Arg	Met	Cys	Thr	Val	Ile	Leu
3.70	9.93	0.78	6.65	4.73	3.97	3.51	8.70
±0.13	±0.24	±0.06	±0.35	±0.11	±0.12	±0.09	±0.21

Phe	His	Asp	Glu	Pro	Ala	Gly	Ser
3.24	1.20	7.76	18.0	4.78	4.10	4.65	7.77
±0.09	±0.03	±0.22	±0.91	±0.22	±0.11	±0.23	±0.17



Means ± SD Relationship between HHQ and HHM (Figure)

Further results indicate a weak relationship between Cys content and improved hoof horn quality, but only in sampling period 2. However, differences between quality classes (I: 7.20 ± 0.05 g Cys/16 g N; all other: 6.57 ± 0.30 g Cys/16 g N; $p < 0.05$) were rather low and overlapped by the period of sampling (1: 5.29 ± 0.35 g Cys/16 g N; 2: 6.65 ± 0.35 g Cys/16 g N).

Conclusion: According to (3) the variation of AA composition in hoof protein indicated a weak connection to horn quality. Factors like feeding and housing conditions or horse breed and age are discussed in the literature, but could not be specified by the current study.

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63.

Crude protein fractions and amino acid content of milk replacers, waste and bulk milk

Vergleich der Rohproteinfraktionen und Aminosäuren von Milchaustauschern, Sperr- und Tankmilch

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Background: Bulk milk (BM) feeding is less popular due to economic reasons. As alternatives waste milk (WM) and milk replacers (MRs) are used, whereby waste milk is obtained from cows during medical treatment and past medical treatment. WM feeding might cause problems in regard of constant protein supply. Commercial MRs contain milk proteins, plant proteins or a mixture of both. Processing of nitrogen sources from milk or plant origin might affect (non-protein) nitrogen sources and especially free amino acids as seen in human formulas (1). The objective of this study was to identify differences in (non-protein) nitrogen sources as well as amino acid content of different milk diets for calves.

Material and Methods: WM and BM samples were obtained on five different German dairy farms, whereby BM samples represented the pool of milk that was eligible for human consumption (n= 5) and WM samples were taken from individual cows (n= 5). MR samples (n=29) were provided by 8 companies and arranged into three groups according to their declared protein sources (Plant (P), Whey (W), Skim milk (S)): PS, WS and PWS). Liquid cow milk samples and powdered MR samples were used for analyses of crude protein and protein nitrogen by VDLUFA methods. Non-protein nitrogen and nitrogen expressed relative to total nitrogen were calculated. Remaining milk samples were lyophilized for amino acid determination. Data were analyzed with one-way ANOVA and post-hoc Tukey's HSD ($p< 0.05$; SAS 9.4).

Results: Total nitrogen was highest in BM, and lowest in all MR ($p< 0.05$). Protein nitrogen was higher in BM and WM than in MR ($p< 0.05$). Non-protein nitrogen was highest in PS-based MR followed by other MR and lowest in BM and WM ($p< 0.05$). Essential amino acids were higher in BM and WM than in MR ($p< 0.05$). The similar pattern was observed for branched chain amino acids (BCAA), whereby within the MRs, PS had lower BCAA than WS ($p< 0.05$).

Conclusion: Based on the assumption that cow milk constitutes the optimal nutritional sources for calves, which seems to be the best assumption as long as there is a lack of nutritional recommendations for young suckling calves, our results indicate that MR composition may not cover the demand. Thus, supplementation of milk replacers with essential and non-essential amino acid mix might be a great strategy to enhance efficiency of milk replacers and optimize growth of calves. Further feeding studies needs to be carried out to investigate effects of amino acid shortage on growth of calves.

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64.

Effects of moderate feed restriction and dietary L-arginine supplementation on the characteristics of an acute innate immune response induced systemic metabolic acidosis in cockerels of a dual-purpose breed

Einflüsse moderater Futterrestriktion und diätetischer L-Arginin-Supplementation auf die Merkmale einer durch akute angeborene Immunantwort induzierte systemische metabolische Azidose bei Junghähnen einer Zweinutzungsrasse

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L-arginine (Arg) is dietary indispensable for chickens and closely related to birds' electrolyte and acid-base balance because of its cationic and alkaline nature as well as its function as precursor of vasodilatory nitric oxides. In general, a physiological electrolyte and acid-base balance is considered to be essential for the appropriate biochemical functioning of metabolic pathways. However, during an acute innate immune response (IIR) the metabolic activity of the immune system rapidly increases and dysregulates the electrolyte and acid-base homeostasis leading to systemic metabolic acidosis (SMA). Considering this tense metabolic state as highly susceptible to nutritional stress, the present study examined potential electrolyte and acid-base balance modulatory effects of surplus dietary Arg in moderate restrictively fed chickens suffering from lipopolysaccharide (LPS) induced IIR.

Methods: A total of 32 one-day-old Lohmann Dual cockerels were commercially reared in a floor-range system for three weeks and in single metabolic cages from day 22 onwards. At day 28 birds were randomly assigned to two diets differing in their Arg concentration only (23.2 % CP; control-diet: 1.37 % Arg; Arg supplemented diet (Arg-diet): 2.04 % Arg; n = 16 birds/diet). In addition, from day 28 to 50 both groups were subject to an *ad libitum* and restrictive regime of feeding, respectively (n = 8 birds/diet*regime). During the entire study the restrictive regime limited birds' feed intake to 75 % of *ad libitum* consumption. On day 50 four cockerels of each group were intramuscularly injected with 2 mg *E.coli* LPS/kg BW (Sigma-Aldrich) as IIR inducer and 1 ml of 0.9 % saline solution (NaCl; B. Braun) as negative control, respectively. At 0, 1, 3, 5, 7 and 24 hours *post injectionem* (h *p. inj.*) rectal body temperature was recorded and 125 µl blood were collected from birds' wing vein via capillary. These samples were immediately analysed for body temperature corrected pH, pCO_2, pO_2, HCO_3^-, lactate, glucose, Na^+, K^+ and Cl^- by a laboratory blood gas analyser (GEM Premier 4000). Based on its diagnostic significance, the anion gap (AG) was further calculated according to $AG = [Na^+ + K^+] - [Cl^- + HCO_3^-]$. Statistical analysis was performed as 2 x 2 x 2 x 6 four-factorial ANOVA (diet, regime, injection and sampling) using SAS procedure MIXED ($p \leq 0.05$).

Results: Regime of feeding and dietary Arg concentration did not affect parameters named above in NaCl treated chickens. Therefore, these birds were just graphically pooled in order to ease readability of Fig. 1. Whereas analysed parameters remained constant after NaCl injection, LPS treated cockerels showed lower blood pH from 1 to 5 h *p. inj.* (Fig. 1a), pO_2 for 1 h *p. inj.* and HCO_3^- from 3 to 7 h *p. inj.* (Fig. 1c), but higher blood lactate concentrations from 1 to 3 h *p. inj.* (Fig. 1b) and pCO_2 for 1 h *p. inj.* than NaCl treated ones before pCO_2 felt below the NaCl treated groups from 3 to 7 h *p. inj.* (Fig. 1d; $p<0.001$). In contrast to latter ones, LPS treated cockerels showed an enlarged AG from 3 to 7 h *p. inj.* ($p<0.001$). Whereas surplus dietary Arg did not modify analysed parameters in LPS treated birds, the moderate feed restriction induced lower blood pH from 1 to 3 h *p. inj.* (Fig. 1a) and HCO_3^- for 1 h *p. inj.* (Fig. 1c) but higher AG for 1 h *p. inj.* compared to *ad libitum* feeding ($p<0.01$).

Conclusions: The LPS induced IIR was accompanied by a strong SMA that resulted from lactate accumulation after anaerobic glycolysis and was partly compensated by respiratory blood CO_2 removal afterwards. Whereas the present study did not reveal any modulatory effects of surplus dietary Arg on bird's electrolyte and acid-base balance, it emphasised the importance of a sufficient dietary supply of energy and nutrients to avoid a metabolic intensification of initial SMA pathology.

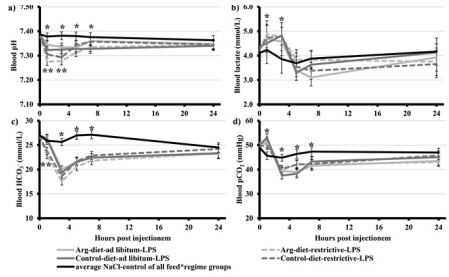

Figure 1. Changes in blood **a)** pH, **b)** lactate, **c)** bicarbonate, and **d)** CO_2 partial pressure of moderate restrictively fed cockerels supplied with additional dietary Arg during 24 hours post LPS or NaCl injection (LSMeans ± SE; n=4). Values with * differ significantly between the NaCl and LPS treated groups ($p < 0.001$). Values with ** differ significantly between restrictively and *ad libitum* fed LPS-groups ($p < 0.01$).

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65.

Effects of increasing dietary Methionine concentrations on the antioxidant status of broilers

Effekte steigender Konzentrationen von Methionin im Futter auf den anti-oxidativen Status von Broilern

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Methionine (Met) is a precursor of cysteine, which is a constituent of glutathione (GSH), an important antioxidant. Major available Met sources include DL-Met (DLM) and DL-2-hydroxy-4-(methylthio) butyric acid (DL-HMTBA) which differ in absorption, transformation to L-Met, and, possibly, in Met trans-sulfuration. We hypothesized that increasing dietary Met positively influences the animal´s antioxidant defence system and investigated the effect of increasing concentrations of both DLM and DL-HMTBA on concentrations of antioxidants and the activity of antioxidant enzymes in broilers.

Methods: 336 one-day old male Cobb 500 broilers were allocated to 42 cages and 7 groups in 3 successive experimental runs. One group of 48 birds (control group) received wheat-soybean meal-based basal diets with concentrations of sulfur-containing amino acids 10% below NRC (1994) recommendations (0.89, 0.74 and 0.69 % Met+Cys of diet DM during days 0-10, 11-21 and 22-35). The other six experimental groups received the basal diets supplemented with 0.10, 0.25 and 0.40% Met either as DLM or DL-HMTBA (equimolar comparison). After 10, 21 and 35 days, body weights and feed intake were recorded, and samples of plasma, liver and jejunum mucosa were collected (n=6 per group). Plasma and tissues were analyzed for GSH (photometry) and α-tocopherol (HPLC) which have antioxidant function, and for thiobarbituric acid reactive substances (TBARS) (photometry) which are by-products of lipid peroxidation. In liver and jejunum mucosa, mRNA abundance (RT-PCR) and activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (photometry) were determined. The data were analysed by ANOVA with the fixed factors Met source, Met concentration, experimental run, and their interactions.

Results: Feed intake and weight gain were higher and the feed:gain ratio lower in all Met-supplemented groups compared to the control group ($P<0.05$), but did not differ between Met sources. Overall, concentrations of α-tocopherol, GSH, and TBARS in plasma, liver and jejunum mucosa were influenced by dietary Met concentration, but not by Met source. Plasma GSH concentrations were similar in all groups at all ages ($P>0.1$). However, GSH in liver increased with increasing dietary Met concentration and was higher by 26, 90, and 84% after 10, 21 and 35 days when diets were supplemented with 0.4% Met as compared to control birds ($P<0.01$). Concentrations of α-tocopherol in plasma were 1.3- to 2.0-fold higher in 10- and 35-day old birds fed Met-supplemented diets compared to control birds ($P<0.001$). In liver and jejunum, concentrations of α-tocopherol were similar in all groups in 21- and 35-day old birds, but in the 10-day old birds, they were 1.7 to 1.9-fold (liver) and 1.5- to 1.6-fold (jejunum) higher when Met was supplemented as compared to the control group ($P<0.001$). Concomitantly, at day 10, plasma TBARS were lower in birds fed Met-supplemented diets (2.0±0.14 to 2.9±0.60 µmol/g triglycerides (TG)) than in those fed the control diet (4.4±1.40 µmol/g TG) ($P<0.001$). In liver and plasma of 21- and 35-day old birds and in the liver of 10-day old birds, the TBARS concentrations were similar in all groups ($P>0.05$). The enzymatic antioxidant system was neither affected by Met concentration nor Met source in the liver. However, in the jejunum mucosa, both mRNA abundance and enzyme activity of SOD, CAT and GPx partly decreased in birds fed Met-supplemented diets compared to control birds.

Conclusion: The supplementation of Met, either as DLM or as DL-HMTBA, increased the antioxidant status in broilers as evidenced by increased concentrations of antioxidants. In addition, lower plasma TBARS concentrations and lower activities of antioxidant enzymes in the jejunum mucosa of birds fed Met-supplemented diets as compared to control birds indicate a lower oxidative burden and a reduced need to counter-act oxidative stress in birds fed Met-supplemented diets.

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66.

Effects of different dietary methionine levels on performance and skin health in growing turkeys

Einfluss unterschiedlicher Methioningehalte im Alleinfutter auf die Leistung und auf die Hautgesundheit junger Puten

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Introduction: Foot pad dermatitis (FPD) is a special challenge in modern poultry production and concerns performance, animal welfare and food safety. Although litter moisture is identified as the main factor to induce FPD (1), it is worth thinking about the role of the essential amino acid methionine (Met) for skin health. The hypothesis was that dietary Met levels higher than current recommended levels favor health of skin, feathers and foot pads, as well as skin barrier function.

Methods: At 14 days of life 216 turkey poults (BUT 6, female) were assigned randomly to 4 treatments (54 turkeys/treatment; with 3 subgroups in each). Feed and water were offered *ad lib*., intake was measured daily on group basis. The pelleted, soybean meal-corn-wheat based diets contained 4.42, 7.30, 9.46, and 12.0 g Met/kg DM, respectively, corresponding to 75, 100, 125, and 150% of current recommendation (2). FPD-Scores (3) and individual body weight were recorded weekly as well as DM content of litter and excreta (per group). After slaughtering on day 43, the skin of the chests was fixed in the vertical arranged Franz Cell Diffusion System between a donor and acceptor chamber. Flufenamic acid (750 µg/ml) was used as donor fluid and after 24 hours the concentration of this donor fluid in the acceptor chamber was analysed by HPLC to measure skin permeability. Furthermore the length of one distinct feather of the wing was measured. Statistical analyses were done by using the SAS® software (PROC GLM/PROC NPAR1WAY).

Results: The following table shows the diet composition and summarizes the most important results:

Group (aimed Met level, %*)	1 (75)	2 (100)	3 (125)	4 (150)
Diet composition in DM**				
XP, g/kg	267	269	269	270
Met, g/kg (in %)	4.42 (60.5)	7.30 (100)	9.46 (129)	12.0 (164)
Cys, g/kg	5.01	5.02	5.11	4.79
MJ ME/kg (calculated)	13.4	13.3	13.4	13.7
Body weight, d 43 (g)	$1431^{a} \pm 207$	$1618^{b} \pm 236$	$1608^{b} \pm 237$	$1581^{b} \pm 228$
DM of "final litter", %	85.3	83.1	75.5	84.7
FPD-Score***, d 42	$1.20^{a} \pm 0.559$	$1.34^{a} \pm 0.550$	$1.46^{a} \pm 0.802$	$1.32^{a} \pm 0.624$
FFA in µg/ml, HPLC****	$9.31^{a} \pm 3.99$	$7.38^{b} \pm 2.46$	$7.01^{b} \pm 2.40$	$9.56^{a} \pm 3.61$
Feather length (mm), d 43	$28.0^{a} \pm 2.67$	$31.5^{b} \pm 3.66$	$32.0^{b} \pm 3.63$	$30.8^{b} \pm 4.35$

*intended, compared to standards; **analysed values; ***low values more favourable (0=healthy; 7= >50% of foot pad necrotic); ****FFA=Flufenamic acid content in acceptor chamber; ^{a,b} indicate sign. differences within a row ($p<0.05$)

Comparing the different treatments group 1 showed lowest body weight and feather length. In all groups a very low FPD Score (a score <2 is without clinical relevance) was found. Skin permeability was lowest in group 2 and 3.

Conclusion: At similar DM contents of the litter (76-85%) there was in general a high foot pad health (FPD Score of 1.2 - 1.5; without any clinical/practical relevance). The recommended dietary Met level allows high growth rate but also a high growth rate of feathers and the intended integrity of the skin including foot pads. The non-supplemented diet (I) resulted in signs of Met deficiency but recommended supplementation (II) ensured a physiological feather growth and skin health.

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67.

Effect of an exogenous protease on growth, pancreas mass and intestinal trypsin activity in chicken fed raw or processed fullfat soybean meal

Einfluss einer zugesetzten Protease auf Wachstum, Pankreasmasse und Trypsinaktivität in Dünndarmabschnitten von Masthähnchen bei Verabreichung roher und behandelter Sojabohnen

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Due to protease inhibitors, raw soybean meal (RSB) in contrast to processed soybean meal (PSB) yields depressed growth, pancreatic enlargement and lower protein digestibility. Hydrothermal processing is applied to prevent these anti-nutritional effects. The question came up if an added exogenous protease could compensate these negative properties of raw soybeans to save energy which is connected with the usual processing of raw beans. The current study aimed to answer this question.

Methods: 240 day-old male meat-type chicken (Ross 308) were randomly allotted to 48 pens for 4 treatments (A: RSB, B: RSB+protease, C: PSB, D: PSB+protease). Diets B and D contained 30,000 U Protease Ronozyme® ProAct/kg diet (DSM). Main dietary ingredients were fullfat soybean meal (33.6 %), wheat (26.5 %) and corn (30.2 %). Hydrothermal processing (A. Kahl, Reinbek) reduced the trypsin inhibitor activity (TIA) from 23 mg/g (RSB) to 3.6 mg/g (PSB). After slaughtering (day 34-37), pancreatic tissue and chyme of duodenum (I) and jejunum (II, from duodenum to Meckel's diverticulum) were sampled. Trypsin activity was measured according to (1) and (2). Trypsin T9201 (Sigma-Aldrich) with ≥ 7,500 BAEE units/mg solid (15,119 units/mg concretely) acted as reference standard. One BAEE unit (benzoyl-L-arginine ethylester hydrochloride) is defined as the increase of the absorption at 253 nm by 0.001 per minute at pH 7.6 at 25°C using BAEE as substrate. Statistical analyses run by two-factorial ANOVA (IBM SPSS Statistics 22).

Results and Discussion: As expected, improved zoo-technical data were observed due to processed soybeans. Protease addition to RSB improved body weight gain and feed conversion ratio and reduced pancreatic mass significantly. A compensation of higher TIA by the added protease failed.

Parameter	Soy bean (SB)	RSB	RSB	PSB	PSB	p-value		
	Protease (P)	without	added	without	added	SB	P	SBxP
Feed intake [g/d]		74.4 ±5.0	77.2 ±5.4	83.5 ±4.8	84.5 ±5.7	<0.0001	0.226	0.567
Body weight gain [g/d]		35.4 ±3.4	39.4 ±2.4	58.8 ±2.4	58.8 ±3.7	<0.0001	0.027	0.028
Feed conversion ratio [g/g]		2.12 ±0.16	1.96 ±0.10	1.42 ±0.07	1.44 ±0.03	<0.0001	0.025	0.006
Pancreatic mass [g/$LM_{kg}^{0.75}$]		5.54 ±1.00	5.07 ±0.63	2.41 ±0.38	2.29 ±0.36	<0.0001	0.027	0.235
Trypsin activity in I [U/g DM]		17,449 ±8,449	20,379 ±5,043	32,948 ±7,329	32,004 ±8,550	<0.0001	0.648	0.374
Trypsin activity in II [U/g DM]		30,788 ±11,245	31,717 ±11,231	67,988 ±11,601	63,250 ±12,291	<0.0001	0.572	0.402

Trypsin activities in both of the intestinal segments (preliminary data) are corresponding to the zoo-technical data, indicating low intestinal protease activity when the dietary TIA is elevated. Exogenous protease did not modulate the observed intestinal trypsin activity. In literature (3) is reported that the presence of exogenous enzymes could even lower pancreatic trypsin secretion.

Conclusion: Adequate processing remains the fundamental prerequisite for an efficient nutrient utilization in chicken soybean based diets and cannot be replaced by the added protease under study.

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(2) Jensen, M. S., Gabert, V. M., Jørgensen, H., & Engberg, R. M. (1997). International journal of pancreatology 21: 173-184.
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68.

Gastro intestinal effects of partly defatted insect meal (*Hermetia illucens)* and micro algae meal (*Spirulina platensis*) as substitute for soybean meal in mixed diets for meat type chicken

Auswirkungen auf den Gastrointestinaltrakt durch den Austausch von Sojabohnenmehl durch teilentfettetes Insektenmehl (Hermetia illucens) oder Mehl der Mikroalge (Spirulina platensis) in Mischfuttern für Masthähnchen

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Insects or Algae are considered as one of the solutions to replace import proteins like soybean meal (SBM) in animal nutrition. The objective of the research, as part of the multidisciplinary project "Sustainability transitions", focused on replacing 50% SBM by partly defatted Hermetia meal (HM) from larvae of the black soldier fly *(Hermetia illucens)* or blue green algae *(Spirulina platensis)* meal (SM) in chicken diets. The current study aimed to evaluate the effects of HM and SM as substitute for SBM diets on praecaecal digestibility (pcD) of CP, factors of intestinal microbiota, mucosal morphometry and microstructure of the small intestine in meat type chicken.

Methods: 180 one-day-old male growing chicken (Ross 308) from a commercial hatchery were randomly allotted to 30 pens (6 birds per pen) for a growth study (starter period 1-21d; grower period 22-34d) with three diets and feed supply on free choice level. The control starter/grower diet (main ingredients: wheat, corn, SBM) contained 39/32% SBM which was replaced in the experimental diets by HM and SM at 50% level making use of a basic AA fortification (AA added: Lys, Met) according to the control diet. After finishing the growth study, 12 (control) and 16 (each for HM and SM) representative broiler were slaughtered. For microbiological studies pooled samples (n=4) were taken from $^{1}/_{3}$ after Meckel's diverticulum until 1 cm before Caecum. Samples were prepared immediately for bacterial growth (CFU total aerob, gram-negatives, clostridia, enterococci and lactobacilli) and parasitological examination. Pooled samples (n=4) from Duodenum until $^{1}/_{3}$ after Meckel's diverticulum for pcD were analyzed for DM, N and TiO_2 as marker. Further 8 birds per diet were slaughtered after 12 hours fastening and utilized for morphometric analysis and histological evaluation of the gut mucosa. Systematic uniform random (SUR) sampling for stereologic investigation of the mucosal surface was carried out from 3 intestinal sections (I1: duodenum, I2: proximal jejunum I3: distal jejunum and ileum). For histology, 5 SUR sub-segments from each section were collected, formalin-fixed (4%), paraffin-embedded and cut into 4 µm sections before routinely stained with hematoxylin eosin. Statistical analysis was performed by One-way ANOVA (SPSS software package Statistics 24) with Tukey-test, Games-Howell-test and Kruskal-Wallis test (ANOVA and multiple comparisons, GraphPad Prism V5) for gut morphometry, respectively.

Results: Bacteria isolated from digesta and total bacterial counts were not significantly influenced by the dietary treatments under study. Parasites were not detected. Final body mass (Control: $2177^{c} \pm 112$g; HM: $1495^{b} \pm 89$g; SM: $1050^{a} \pm 69$g) and pcD of CP (Tab.) were significantly lower with diet SM. Intermediate results yielded diet HM, but pcD tended to be superior to the control. As compared to control, morphometric analysis of the small intestine revealed a highly significant increase ($P<0.0001$) of the relative mucosal surface in all gut sections due to diet SM, most pronounced in segments I1 and I2. Similar observations, but to a lower extent, resulted with diet HM and differences between HM and SM were only significant in I1 ($P<0.05$). As compared to control and HM, preliminary histological data showed conspicuous increase of the mucosal surface due to enlargement of villi with diet SM. Final stereologic evaluation is still in progress. Some evidence of increased influx of inflammatory cells into *Lamina propria* due to diet SM needs further quantitative analysis.

Diets	Control diet (n=4)	Diet HM (n=4)	Diet SM (n=4)
Praecaecal CP digestibility (%)	$64.63^{b} \pm 5.2$	$65.25^{b} \pm 4.9$	$49.03^{a} \pm 6.2$

Mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$).

Conclusion: Results indicate that insect meal based diets yielded superior pcD of CP and only minor effects on intestinal morphometry. In contrast, algae meal provided low pcD and some modification of intestinal microstructure which need further attention in ongoing experiments.

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69.

Response of meat type chickens due to amino acid optimization of mixed diets with 50% replacement of soybean-meal by partly defatted insect meal *(Hermetia illucens)* or meal from micro algae (*Spirulina platensis*)

Reaktionen von Masthähnchen auf eine Aminosäurenoptimierung des Mischfutters bei 50% Austausch von Sojaextraktionsschrot gegen teilentfettetes Insektenmehl von Hermetia illucens oder Mikroalgenmehl von Spirulina platensis

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Alternative protein sources, such as insects or algae meals are in special focus of animal nutrition in order to replace soybean meal (SBM). As part of the multidisciplinary project "sustainability transitions" this study investigated effects of replacing SBM by partly defatted larvae meal from the black soldier fly, *Hermetia illucens* (HM) or the blue green algae *Spirulina platensis* (SM) in broiler diets. Both of the alternative protein sources have high protein contents (60.84 and 58.76 g/kg DM, respectively) and a balanced amino acid (AA) composition. The aim of the study was to evaluate the potential of HM and SM as an alternative protein source for SBM diets for meat type chicken with 50% replacement of SBM during the fattening period.

Methods: 288 one-day-old male growing chickens (Ross 308) from a commercial hatchery were randomly allotted to 48 pens (6 birds per pen) for a growth study (starter period 1-21d; grower period 22-34d) with five diets and feed supply on free choice level. The control starter/grower diet (main ingredients: wheat, corn, SBM) contained 39/32% SBM. The experimental diets replaced 50% of SBM by the alternative proteins under study, both on a basic (diets HM, SM; AA added: Lys, Met) and an advanced level of AA fortification (diets HM+AA, SM+AA; AA added: Lys, Met, Thr, Arg, Val) to achieve up to 105% of the AA supply of control diet (e.g. Starter: Lys:Met:Thr:Arg:Val 100%:39%:62%:113%:74%) according to current ideal AA ratio (IAAR) recommendations (1). Response of chicken was evaluated both by zoo-technical parameters which were under weekly control (growth, feed intake, FCR, mortality) and protein utilization (PNu_{std} as corrected for equal daily N intake of 3000mg/$BW_{kg}^{0.67}$) as measured by whole body N analyses at the end of the study. One-way ANOVA (SPSS software package Statistics 24) connected with Tukey-test and Games-Howell-test identified significant differences between treatments ($p \leq 0.05$).

Results: Summarized results (Table) indicate that diet HM+AA yielded both superior growth of body mass and significantly improved FCR as compared to the control diet. Diets on the basic level of AA fortification (HM, SM) led to significant depression of growth, feed intake, FCR, and protein utilization, respectively. Acceptance of diet SM was lower ($p \leq 0.05$) as compared to diet HM. As compared to basic level, AA fortification improved all parameters significantly.

Table: Summarized results (Growth period 1-34d) of the study (Mean ±SD)

Diets	Control (n=12)	HM (n=9)	SM (n=9)	HM+AA (n=9)	SM+AA (n=9)
Final body mass (g)	2177^{c} ±112	1495^{b} ±89	1050^{a} ±69	2319^{d} ±114	2118^{c} ±122
Feed intake (g/d)	87.3^{c} ±4.7	75.4^{b} ±6.2	58.8^{a} ±3.4	87.1^{c} ±5.5	86.0^{c} ±5.8
Feed conversion ratio (g/g)	1.35^{b} ±0.04	1.72^{c} ±0.17	1.89^{c}* ±0.11	1.26^{a} ±0.04	1.37^{b} ±0.02
PNu_{std} (%)	62.46^{c} ±1.32	48.46^{a} ±3.66	46.11^{a}* ±2.08	62.97^{c} ±1.35	60.07^{b}* ±0.67

Means in the same column with different superscript letters differ significantly ($p \leq 0.05$) *n=8

Conclusion: Both_partly defatted meal of *Hermetia illucens* and algae meal of *Spirulina platensis* are promising alternative protein sources in chicken diets when the dietary AA balance is well adapted to the IAAR by an enlarged range of supplemented feed AAs.

Wecke C. and Liebert F. (2013): Animals 3: 558-573.

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70.

Crude protein and ash contents of feathers and feather-free body of actual meat type chicken (Ross 308) depending on age and sex

Rohprotein- und Rohaschegehalte in Federn und im federfreien Körper von aktuellem Mastgeflügel (Ross 308) in Abhängigkeit von Alter und Geschlecht

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Results about the body composition of actual broiler genotypes are important for valid evaluation of both optimal protein and amino acid supply by factorial methods and consequently also for further minimizing of N excretion according to the future demands. The present study aimed to investigate if whole body composition data based on fractional analysis of feather and feather-free body depends on age and sex of modern meat type chickens needs to be revised.

Methods: Two growth studies with 180 male and female (1:1) meat type chickens (Ross 308) were conducted. The starter (1- 22d) and grower diets (22-36d) utilized a constant mixture of corn, wheat, soybean meal, soybean protein concentrate and crystalline amino acids (AA). Diets were formulated to provide nutrient and energy supply according to current recommendations. The dietary AA composition was adjusted close to the ideal AA ratios [1]. Day old chickens were randomly allotted to 30 floor pens (5birds/pen; 15 pens/gender). Additionally, 15 day old male and female birds (3 samples of 5 birds/sex) were euthanized by CO_2 inhalation following 24h fasting. Feathers were manually removed and quantified. Sampling was repeated weekly up to the end of the study. In feathers and feather-free body the dry matter (DM), N and ash contents were analysed according to standard procedures of VDLUFA. One-way and two-way ANOVA (SPSS software package) connected with Tukey-test were utilized to identify significant differences (*p<0.05; **p<0.01; ***p<0.001; ns = not significant) between variables age (A) and sex (S), respectively.

Results and Discussion: Due to the utilized common factor (N x 6.25) the crude protein (CP) content of the feather fraction as N-rich keratin compound is near at 100% very high but degreased significantly with increasing age (Table). Dependent on sex, no significant effects on feather CP content were observed. The CP content both of the feather-free body and empty body was significantly decreased as age of birds was enhanced. In addition, the average feather percentage increased non-linearly from approximately 2% at the end of the 1st week to approximately 4% at the end of the 5th week of age and was significantly higher in female (f) *vs.* male (m) birds [2]. Generally, in all age periods significantly lower CP content was observed in the body of female birds as compared to male counterparts. The ash content of the feathers was very low (1.0-2.5% of DM), whereby no sex- but age-dependent differences were found. De-feathered as well as empty body of female birds yielded a linear decline of ash with increasing age whereas in male birds a non-linear course was observed with lowest ash content at 15d.

Age (d)	Feathers (% DM)				Feather-free body (% DM)				Empty body (% DM)			
	CP		Ash		CP		Ash		CP		Ash	
	m	f	m	f	m	f	m	f	m	f	m	f
1	100	102	1.0	1.1	71	70	9.4	9.4	75	73	8.4	8.6
8	101	99	1.6	2.0	68	62	8.6	8.3	69	64	8.2	8.0
15	96	97	2.6	2.0	65	62	8.3	8.3	66	63	8.1	8.0
22	98	97	2.1	1.8	65	61	8.6	8.3	67	62	8.3	7.9
29	99	98	1.7	1.5	63	59	8.7	8.1	65	61	8.4	7.6
36	99	99	1.5	1.4	64	56	8.7	7.6	66	60	8.2	7.2
Age	*	***	***	***	**	***	**	***	***	***	ns	***
Sex	ns		ns		**		*		**		**	
AxS	ns		***		*		**		ns		***	

Conclusions: According to the observed variation of nutrient content in feathers and the featherless body, effects on the whole body composition based on valid regression analyses depending on age period and sex of growing chickens have to be considered. These factors need more attention in requirement studies, namely when they are based on factorial approaches.

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(2) WECKE, C et al. (2015): Tagungsband 13. Tagung Schweine- und Geflügelernährung, Lutherstadt Wittenberg, 40-42.

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71.

Influence of grape seed and grape marc meal extract on the hepatic transcript profile and the plasma lipid profile of early lactating dairy cows

Einfluss von Traubentresterextrakt auf das Transkriptprofil der Leber und das Lipidprofil des Blutplasmas von frisch laktierenden Milchkühen

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The first weeks (wk) after parturition are the most critical period in the life of dairy cattle due to the high risk to develop metabolic and infectious diseases. Despite ongoing discussion about the underlying reasons, systemic inflammation has been increasingly recognized as an important contributing factor, because chronic inflammation, even if only low grade, is detrimental for health and performance of cows. Recently, feeding of polyphenol-rich grape seed and grape marc meal extract (GSGME) to transition dairy cows was reported to increase milk yield, but the underlying reasons remained unclear.

Question: As polyphenols exert a broad spectrum of biological effects, we hypothesized that feeding of GSGME modulates critical metabolic and inflammatory signaling pathways in the liver which could account for the positive effects of GSGME in dairy cows. In order to identify these pathways, we performed genome-wide transcript profiling in the liver of cows fed GSGME during the transition period. In addition, we carried out plasma lipid profiling in order to detect potential changes in critical lipid species known to act as signaling molecules.

Methods: 28 primi- and multiparous Holstein cows with an average parity number of 2.8 were assigned into 2 experimental groups: control group (n = 14) and grape seed grape marc meal extract (GSGME) group (n = 14). From wk 3 ante partum until calving, a total mixed ration (TMR) was fed to meet the demand of net energy and crude protein of a dry cow with a BW of 650 kg and an assumed dry matter intake (DMI) of 12 kg/d. After calving, all animals were offered a basal TMR calculated to meet the demand of net energy and crude protein for producing 34 kg of milk, with an assumed daily DMI of 22 kg. The TMR of the GSGME group was supplemented with 1% (of DM) of commercially available polyphenol-rich GSGME (Antaox, Dr. Eckel, Niederzissen, Germany). The TMR of the control group was supplemented with 1% of wheat bran for an energetic adjustment. Blood and liver samples were collected at 1 wk p.p. from the *vena caudalis* and from the right liver lope, respectively. Plasma obtained from blood by centrifugation of n = 14 cows/group was used for determination of selected acute phase proteins (APP) and extraction of plasma lipids and subsequent plasma lipid profiling using direct flow injection electrospray ionization tandem mass spectrometry (ESI-MS/MS). For microarray analysis, total RNA isolated from liver samples of n = 6 animals per group was used for hybridization to the Affymetrix GeneChip Bovine Gene 1.0 ST array representing approximately 23,000 bovine transcripts. Transcripts were defined as differentially expressed when the fold-change between GSGME group and control group was >1.3 or <-1.3 and the P-value of an unpaired Student´s t-test for each transcript was < 0.05.

Results: Transcriptome analysis of the liver revealed 207 differentially expressed transcripts, from which 156 were up- and 51 were down-regulated, between GSGME group and control group. Gene set enrichment analysis of the up-regulated transcripts showed that the most enriched gene ontology (GO) biological process terms are dealing with cell cycle regulation and the most enriched Kyoto Encyclopedia of Genes and Genomes pathways were p53 signaling and cell cycle. Functional analysis of the down-regulated transcripts revealed that a great part of these genes are involved in endoplasmic reticulum (ER) stress-induced unfolded protein response (UPR) and inflammatory processes. Accordingly, protein folding, response to unfolded protein, unfolded protein binding, chemokine activity and heat shock protein binding were identified as one of the most enriched GO biological process and molecular function terms assigned to the down-regulated transcripts. In line with these data plasma concentrations of the APP serum amyloid alpha and haptoglobin were reduced in the GSGME group compared to the control group. Analysis of plasma lipid species revealed no differences in the concentrations of individual species of major and minor lipid classes between both groups of cows.

Conclusion: Genome-wide transcript profiling of the liver indicated that a polyphenol-rich feed component is able to inhibit ER stress-induced UPR and inflammatory processes, both of which are considered to contribute to liver-associated diseases and to impair milk performance in dairy cows, in the liver of dairy cows during early lactation.

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72.

Fermentation characteristics along the equine gastrointestinal tract after feeding of Jerusalem artichoke meal

Fermentationscharakteristika entlang des Verdauungstraktes von Pferden nach der Fütterung von Topinamburmehl

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Prebiotic fructooligosaccharides (FOS) and inulin (INU) are supposed to stabilize the hindgut microbiota and have thus the potency to prevent gastrointestinal disorders in horses (1, 2). Nevertheless, evidence exists that fructans of this type are started to be fermented already in the foregut (3). We hypothesized that prebiotic quantities of FOS+INU increases the concentrations of fermentation products in both, the horses' foregut and hindgut. Aim of the study was to investigate whether a permanent supply of prebiotic doses of FOS+INU *via* Jerusalem artichoke meal (JAM) affects fermentation products and pH values in different parts of the gastrointestinal tract of horses.

Methods: During 3 weeks, 2 x 6 adult healthy warmblood horses (10 mares, 1 stallion, 1 gelding; body weight [BW] 534 ± 64.5 kg) were fed crushed oat grains (1.2 g starch/kg BW d^{-1}) and meadow hay (1.5 kg/100 kg BW d^{-1}) in 2 equal meals per day. Additionally, they received either 0.15 g FOS+INU/kg BW d^{-1} *via* JAM or an equal amount of maize cob meal without grains as control (CON). At the periods end the horses were euthanized ≈ 1 h after they had received ½ of the daily concentrate ration. Digesta was sampled from the stomach (pars nonglandularis, PN; pars glandularis, PG), small intestine (SI) caecum (CAE) and colon (ventrale [CV], dorsale [CD] and transversum [CT]) and analyzed for pH values, ammonia (Conway), total and individual short chain fatty acids (SCFA; gas chromatography) and D-/L-lactate (HPLC). Data were analyzed by PROC MIXED (two-way ANOVA with repeated measures; SAS, version 9.4) at a significance level of $P < 0.05$.

Results: JAM did not significantly affect ($P > 0.05$) any of the measured fermentation products or the pH values along the gastrointestinal tract, with exception of ammonia in the PG (JAM > CON; $P < 0.05$; tab.). JAM increased the concentrations of total SCFA and from this particularly *n*-butyrate as well as both lactate isomers in the stomach numerically ($P > 0.05$). In the hindgut, a more pronounced stimulation of the microbial activity by JAM *vs* CON was limited to the CV indicated by slightly higher concentrations of total SCFA with emphasis on *n*-butyrate and ammonia ($P > 0.05$).

Conclusion: The results from this study confirm the assumption that FOS+INU from JAM are already started to be fermented in the foregut and particularly in the stomach of horses which needs to be critically evaluated with regard to the stomach health. On the contrary, the *per se* desired stimulation of microbial fermentation in the large intestine by FOS+INU was clearly lower than expected. It needs to be taken into account, however, that the majority of results interpreted here had a numeric but not statistical power only, due do the high inter-individual variation.

item	group	PN	PG	SI	CAE	CV	CD	CT
SCFA	CON	57.3 ± 12.9	22.3 ± 8.7	5.3 ± 1.9	167.2 ± 18.4	195.4 ± 19.1	163.3 ± 17.8	69.3 ± 11.1
	JAM	62.3 ± 12.9	35.6 ± 8.7	4.1 ± 1.9	128.0 ± 18.4	217.7 ± 19.1	167.6 ± 17.8	82.1 ± 11.1
ammonia	CON	9.7 ± 1.4	6.5[a] ± 0.5	8.6 ± 1.3	2.7 ± 0.6	7.6 ± 2.5	10.2 ± 2.1	9.9 ± 1.1
	JAM	12.7 ± 1.4	8.2[b] ± 0.5	7.1 ± 1.3	2.4 ± 0.6	13.4 ± 2.5	8.8 ± 2.1	9.7 ± 1.1

LSmeans ± SE; all items in mmol/L; [a,.b] superscripts in one row indicate significant differences ($P < 0.05$)

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73.

The combination of high dietary zinc oxide and chlortetracycline amplifies enterobacterial antibiotic resistance genes in weaned pigs

Die Kombination hoher Zinkdosierung und Chlortetracyclin verstärkt Antibiotikaresistenzgene von Enterobakterien in abgesetzten Ferkeln

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Question: Dietary zinc oxide in high doses (up to 3g/kg feed) is used in many countries in piglet nutrition to successfully combat *E. coli* induced post weaning diarrhea in piglets. Similarily, a combination with antibiotics is common, for instance in Asia and the Americas.
The antibiotic effect of dietary ZnO by itself leads to a drastically modified intestinal microbiota in weaned pigs with possible detrimental effects later in life and has been shown to increase antibiotic resistance in enterobacteria. The addition of antibiotic growth promoters is also thought to increase bacterial antibiotic resistance. Thus, the aim of this study was to closely monitor the development of enterobacterial antibiotic resistance genes in pigs directly after weaning to investigate possible additive effects of dietary zinc oxide and chlortetracycline.

Material and methods: Weaned pigs (n=10 per group) were either fed diets with 110 mg/kg or 2400 mg/kg of a commercial zinc oxide preparation with or without addition of 300 mg/kg chlortetracycline (CTC). Fecal samples were taken at 0, 2, 4, 7 and 14 days of the trial and subsequent DNA extracts were amplified by qPCR assays for a number of enterobacterial antibiotic resistance genes. Additionally, data on 16S rRNA gene copy numbers from the *Escherichia* group were used to estimate the impact of the additives on the development of enterobacterial antibiotic resistance. Data was analysed by the Kruskal-Wallis test, followed by Mann-Whitney U test, where appropriate.

Results: A clear effect of high dietary zinc oxide as well as of CTC was visible regarding performance (data not shown). As expected, the reduction of *Escherichia* correlated well with zinc oxide or CTC intake i.e. a significant reduction for *Escherichia* was visible starting 4d after weaning, when feed intake increased ($p<0.05$ for high ZnO, CTC and high ZnO&CTC). However, only marginal changes were visible in the low ZnO trial group. The enterobacterial resistance gene against sulfonamides (*sul*1) was significantly lower in the low ZnO trial group compared to all other trial groups after 4d ($p<0.05$). An enterobacterial gene for tetracycline resistance (*tet*A) also decreased in the low ZnO trial group until day 4 of the trial, while the high ZnO trial group showed a slight increase in *tet*A copy numbers throughout the trial. However, the combination of CTC with low or high dietary zinc led to a significant increase in *tet*A copy numbers from day two onward ($p<0.05$). Furthermore, the ratio of *tet*A copy numbers to 16S rDNA copy numbers of *Escherichia* spp. showed after 4 and 6 days of the trial increasing ratios in the order of low ZnO < high ZnO < low ZnO & CTC < high ZnO & CTC. Most strikingly however, the ratios of *sul*1 to *Escherichia* spp. followed the same course as for tetracycline ratios, although no sulfonamide antibiotic was administered.

Conclusions: This study has shown that there is an additive effect of high dietary ZnO and chlortetracycline regarding enterobacterial resistance against tetracycline. Furthermore, there are strong indications that enterobacteria resistant against other antibiotics (here sulfonamides) may also gain a colonization advantage by the combination of ZnO and CTC.

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74.

Influence of *myo*-inositol or phytase supplements on $InsP_6$ disappearance and *myo*-inositol concentration in crop, lower ileum, and blood of broilers

Einfluss von Zusätzen von myo-Inositol oder Phytase auf den $InsP_6$-Abbau und die myo-Inositol-Konzentration in Kropf, hinterem Ileum und Blut von Broilern

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In a recent study, phytase and *myo*-inositol supplementation to P and Ca deficient diets showed similar improvements of broiler's performance [1]. How *myo*-inositol instead of phytase supplementation affects the inositol hexakisphosphate ($InsP_6$) breakdown and *myo*-inositol in different segments of the digestive tract and blood remained unanswered. Therefore, this study distinguished the effects of a supplementation of free *myo*-inositol and graded levels of phytase on the disappearance of $InsP_6$ and the *myo*-inositol concentration in crop, ileum and blood plasma.

Methods: At day 1 of the trial, 40 pens with 11 Ross 308 broiler hatchlings each were randomly allocated to 5 dietary treatments. The basal diet (BD; wheat, soybean meal, corn) contained adequate nutrient levels according to the recommendations of the breeding company (8.7 g Ca and 6.9 g P per kg grower diet). The Phy diets were reduced in P and Ca levels (7.1 g Ca and 5.4 g P per kg) and supplemented with 500, 1500 or 3000 FTU/kg of a modified *E.coli* derived 6-phytase (Quantum™ Blue, AB Vista). Diet MI was the BD supplemented with 4.0 g *myo*-inositol/kg. At day 22, blood samples were collected from one animal per pen after decapitation. Digesta from the crop and a defined section of the lower ileum of all animals was obtained, pooled on a pen basis and freeze dried. Fluoride plasma samples were analyzed for *myo*-inositol. Digesta samples were analyzed for $InsP_6$ by HPIC, *myo*-inositol by GC-MS and titanium dioxide (TiO_2), which served as the indigestible marker. A one-way ANOVA was carried out using SAS 9.3. Significance was declared at $P < 0.05$.

Results: No significant effects on average daily gain (ADG) were detected (Table). However, the gain:feed ratio (G:F) was significantly increased by phytase and *myo*-inositol. *Myo*-inositol did not significantly affect the disappearance of $InsP_6$ in crop and up to the end of the ileum. Addition of phytase significantly increased $InsP_6$ disappearance in both segments with a higher disappearance in Phy1500 than in Phy500, but no further increase by Phy3000. *Myo*-inositol concentration in crop and ileum was increased by phytase and was even higher in MI fed birds. In the ileum, the *myo*-inositol concentration increased with phytase dose. The *myo*-inositol concentration in plasma was increased by *myo*-inositol and phytase addition, but no significant differences between the phytase levels were found.

Table: Performance, $InsP_6$ disappearance and *myo*-inositol concentration in crop, ileum and plasma

	BD	Phy500	Phy1500	Phy3000	MI	SEM
ADG, g/d	53	53	53	52	53	0.81
G:F, g/g	0.82^{b}	0.84^{a}	0.84^{a}	0.84^{a}	0.84^{a}	0.004
$InsP_6$ disappearance crop, %	30^{c}	47^{b}	56^{a}	58^{a}	31^{c}	4.50
Precaecal $InsP_6$ disappearance, %	31^{c}	70^{b}	93^{a}	94^{a}	28^{c}	2.70
Myo-inositol crop, g/kg DM	0.21^{d}	0.24^{c}	0.25^{c}	0.43^{b}	2.19^{a}	0.03
Myo-inositol ileum, g/kg DM	0.59^{e}	1.93^{d}	3.29^{c}	4.28^{b}	6.47^{a}	0.20
Myo-inositol plasma, mmol/L	0.23^{c}	0.32^{b}	0.36^{b}	0.33^{b}	0.52^{a}	0.03

$^{a\text{-}e}$Different superscript letters within a row indicate significant differences ($P < 0.05$).

Conclusions: The results confirmed that phytase and *myo*-inositol can improve feed efficiency. *Myo*-inositol seemed to have no effect on gastro-intestinal phytate degradation. Phytase addition increased *myo*-inositol concentration in crop, ileum and plasma by dephosphorylating phytate. Further studies are needed to understand which *myo*-inositol levels in the digesta correspond to higher blood levels and which blood *myo*-inositol levels affect metabolic traits.

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75.

Investigations on the effects of dietary cereal type, crude protein content and butyrate supplementation on carcass composition of broiler chickens

Untersuchung der Wirkung des Getreidetyps, des Rohproteingehaltes und der Butyratsupplementierung auf die Körperzusammensetzung bei Broilern

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Broiler chickens play remarkable role in food production; therefore, investigations on the effect of different nutritional factors on carcass composition are of great importance. Several dietary components, such as the cereal types, crude protein content, amino acid profile, and certain feed additives, for instance organic acids, oils and extracts of plant origin may alter carcass traits and meat composition. Among others, the short chain fatty acid butyrate is a widely used alternative growth promoter in poultry nutrition, which may also act on carcass characteristics. As the possible influence of dietary cereal type, dietary crude protein content and butyrate supplementation on meat quality is not yet fully elucidated, we aimed to assess these factors in our study.

Methods: Ross 308 broiler chickens were randomly allocated to eight groups (n=10/group). Four of them were fed with wheat-based diet, rich in soluble non-starch polysaccharides (NSP) and supplemented with xylanase-glucanase enzyme mixture, while the others were reared on maize-based diet, representing lower dietary NSP level. Wheat-based diet with higher NSP content provided more substrates for the caecal microflora, stimulating the endogenous production of short chain fatty acids, particularly that of butyrate. Crude protein content of the diets was set to the requirements of the appropriate dietary phase (normal protein = NP) or reduced by 15% in certain experimental groups, the latter supplemented with limiting amino acids to avoid the diminishing effects of inadequate limiting amino acid supply (low protein = LP). Feed of the animals was formulated with the application of sodium (n-)butyrate or without it in the control groups. On week 6, live weight and following slaughtering, carcass weight as well as weights of pectoral muscle, thighs, liver, heart, spleen and abdominal fat were measured. Further, chemical analysis of pectoral and femoral muscle was carried out, revealing the dry matter, protein and lipid content. Effects of wheat-based diet compared to maize-based dietary groups, and differences between NP and LP as well as between control and butyrate-supplemented groups were studied by multi-way ANOVA, using the R 3.2.2 software; differences were considered as statistically significant if $P<0.05$.

Results: Live weight was significantly elevated by LP diet and butyrate supplementation ($P<0.05$), while carcass weight was significantly increased only by the lower dietary crude protein content ($P<0.05$). Relative weight of pectoral muscle increased in LP groups ($P<0.05$); however, relative liver weight was decreased by wheat-based diet ($P<0.001$) and increased by butyrate application ($P<0.001$). The relative mass of heart and spleen diminished ($P<0.001$), while that of abdominal fat increased in groups fed with diets containing lower crude protein level ($P<0.05$). Relative weight of thighs was not influenced by any of the investigated nutritional factors. Concerning the chemical analysis of meat composition, dry matter content of femoral muscle was decreased by wheat-based ($P<0.05$), but was increased by LP diet ($P<0.01$). Protein content of femoral muscle diminished ($P<0.001$) and fat content elevated in LP groups ($P<0.001$). Butyrate supplementation decreased the protein content ($P<0.001$) and increased the fat content ($P<0.001$) of femoral muscle.

Conclusion: According to our results, the investigated factors significantly influenced carcass composition of broiler chickens. The increasing action of LP diet on live weight and carcass weight might be explained by the applied limiting amino acid supplementation, providing more limiting amino acids in free form. Literature data indicated that reduced crude protein content of diet decreased nitrogen excretion, however, according to our results, increased the abdominal fat mass, possibly due to the elevated dietary cereal content of isocaloric LP diets. Significantly lower giblet weights of chickens kept on LP diet are suggested to be in connection with the priority of developing breast meat even under suboptimal dietary conditions. Chemical composition of femoral muscle changed in case of various nutritional factors, while that of pectoral muscle remained unchanged, which might be in connection with the distinct metabolism of the two muscles. Increasing effect of butyrate supplementation on the fat content of thighs may contribute to improve meat quality; therefore it can be advantageous from food quality point of view.

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76.

Effects of polyphenol product on performance and blood parameters in heat stressed broilers

Einfluss eines Polyphenolproduktes auf Leistung und Blutparameter bei hitzegestressten Broiler

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Introduction: During the summer months, high temperatures and a high stocking rate cause heat stress, which in consequence decreases the animals' productivity and increases morbidity and mortality. Polyphenols have been shown to positively influence oxidative status and immune system of animal, consequently leading to increased animal performance (1).

Material and methods: 180 1-day old broiler chickens were randomly allocated to 3 treatments with 4 repetitions of 15 birds each. Treatments were T1 - control; T2 - control plus heat stress (HS); T3- control plus heat stress and addition of a polyphenol product (PP) with the main active ingredients tumeric and green tea extracts (Spicemaster CP alpha; Kaesler Nutrition GmbH, Cuxhaven, Germany) with 300 mg/kg feed. Birds were fed a starter diet form day 1-14 and a grower diet from day 15-42 according to GfE recommendations (2). Birds of T2 and T3 were exposed to acute heat stress (30°C) from day 21-42. At day 42, birds were sacrificed using CO2 asphyxiation. Blood samples from vena cutanea were taken from 12 birds per treatment into plain and heparinized ADTA containing tubes for determination of hematological parameters and biochemical parameters as well as liver enzymes. Data were analyzed by using SPSS based on one way ANOVA. All treatment least squares were compared by the Tukey test.

Results: Already before heat stress period, birds receiving the polyphenol product showed improved performance during following periods: weight gain (+10%; 118.4 *vs.* 106.6 g/day, T3 *vs.* T1, respectively; P=0.056) from day 1-7, feed conversion ratio (FCR) (-7.1%; 1.25 *vs.* 1.36 g/day, T3 *vs.* T1, respectively; P=0.018) from day 8-14. Heat stress significantly ($p < 0.001$) reduced body weight gain in the heat exposed group during the whole stress period from day 22 - 42. Feeding the PP numerically increased weight gain under heat stress compared to heat stressed birds without PP (5.1%; 409.3*vs.* 388.4 g T3 *vs.* T2; p=0.303) from day 22-28. Additionally, feeding the PP resulted in improved oxidative status as indicated by reduced malonyl dialdehyde (MDA) compared to HS without PP. Supplementation of PP numerically decreased alanin-aminotransferase (ALAT) and cortisol and significantly reduced interleukin 6 (IL6) compared to HS group (table 1).

Table: Blood parameters of broilers at day 42. [a,b] Values in the same row with no common superscript are significantly different (P<0.05).

Parameter	T1 Control	T2 Control + HS	T3 HS + PP	SEM	P-value
MDA (nmol/ml)	1.52 [ab]	1.58[a]	1.48[b]	0.017	0.009
ALAT (µkat/l)	0.11	0.14	0.12	0.005	0.096
IL 6 (pg/ml)	176.3 [a]	266.2[b]	194.7[c]	12.540	0.005
Cortisol (ng/ml)	46.3[a]	83.0[b]	64.7[ab]	3.886	<0.001

Conclusion: The results of this study indicate that adding the PP product supports high performing broilers not only during periods of heat stress. However, the mode of action has to be clarified in further studies.

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77.

Energetic benefits of supplementing an endo-1,4-arabinoxylanase/endo-1,4-ß-glucanase preparation to wheat-based diet for broiler chickens

Zulage einer Endo-1,4-Arabinoxylanase/Endo-1,4-ß-Glukanase Formulierung zur Weizen-basierten Ration auf die Energie- und Nährstoffversorgung junger Masthähnchen

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The study was conducted to evaluate the effect of a Non-Starch-Polysaccharide-hydrolysing enzyme preparation (NSPE), containing endo-1,4-arabinoxylanase and endo-1,4-ß-glucanase, on diet AME in male broiler chicken, while also monitoring performance, ileal digesta viscosity, and apparent total tract digestibility (ATTD) of main nutrients.

Methods: A total of 192 one day old male broiler chickens (Ross 308) allocated to 48 cages were used. Pelleted feed and water were provided *ad libitum* during the whole 24 days of the trial. Two treatments were tested: a negative control diet (NC) based on 63% wheat and 21% soybean meal (2950 kcal/kg, 1.07% lysine and 0.41% methionine), and an enzyme diet (EF) supplemented with the NSPE. All diets contained 0.5% TiO_2 as a marker. Enzyme activity in the EF feed was analysed to be 590 TXU** and 270 TGU** per kg. Performance parameters were measured per replicate at days 14 and 24 of the trial. From day 21-23 representative excreta samples from each replicate were collected for ATTD determination. At day 24, birds were sacrificed and the upper half of the ileum section was flushed to remove the digesta for viscosity measurement. Data were analysed by ANOVA and the experimental unit was the replicate, consisting of 3 adjacent cages of 4 (0-14 days) or 2 (15-24 days) chickens.

Results: NSPE addition increased BWG (pTable 1). Digesta viscosity was reduced by NSPE supplementation from 4.49 (NC) to 2.58 mPs (EF) (pTable 2). ATTD was improved in EF compared to NC for all parameters but protein and crude fiber (CF) and for AME ($p<0.05$).

Table 1: Daily BW gain (BWG), feed intake (FI) and feed conversion ratio (FCR)

Diet	BWG	FI	FCR
	g/d	g/d	feed/gain
NC, day 0-14	24.9[b]	34.9	1.400
EF, day 0-14	26.5[a]	35.3	1.332
NC, day 15-24	69.8	109.9	1.574[a]
EF, day 15-24	70.2	107.5	1.543[b]

*[a-b]Means within columns and in same time period without common superscript differ significantly (p

Table 2: ATTD for dry matter (DM), organic matter (OM), energy, nitrogen, fat, crude fibre (CF) and diet AME

Diet DM OM	Energy Nitrogen	Fat CF	AME
% %	% %	% %	Kcal/kg
NC 65.4[b] 67.6[b]	69.0[b] 84.3	77.2[b] -6.9	2842[b]
EF 68.4[a] 70.6[a]	72.3[a] 84.3	82.0[a] -14.7	2979[a]

*[a-b]Means within columns without common superscript differ significantly (p

Conclusions: Supplementation of NSPE to a wheat-based diet fed to broiler chickens improved diet AME by improving ATTD of the main nutrients. It increased growth performance and reduced ileal digesta viscosity. NSPE might be a valuable tool to increase the nutritive value of wheat-based diets for broiler chickens.

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**Thermostable Xylanase Unit (TXU), Thermostable Glucanase Unit (TGU)

78.

Influences of different dietary levels of Mannan-degrading enzymes on performance, on viscosity in small intestine contents, as well as on histology of small intestine in broilers

Einfluss unterschiedlicher Gehalte von Mannanase im Alleinfutter für Broiler auf die Leistung, auf die Viskosität im Dünndarmchymus sowie auf die Histologie des Dünndarms von Broilern

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Non-starch polysaccharides (NSPs) can be divided into soluble and insoluble fibres. The soluble NSPs are able to impair the utilisation of nutrients by increasing the digesta viscosity [1, 2]. Galactomannan is the most important soluble NSP in soybean meal (SBM), the common protein source in broiler diets. By dietary Mannan-degrading enzymes this negative effect should be minimized. The hypothesis of this study was that the viscosity and utilization of energy and nutrients are improved by the use of Mannanase (M) in broilers' diets. Subsequently an improved performance of broilers was expected. In addition histological parameters of the small intestine were measured to look at morphological changes of the small intestine, maybe related to the use of enzymes.

Methods: 264 Ross 308 broilers (1 day old, mixed sex) were randomly assigned to 4 treatments with 3 replications each. Each treatment consisted of 2 groups à 17 broilers and 1 à 32 birds. Water and Feed were offered always ad libitum. The wheat based diet contained 30% SBM, 2% soybean oil and 4% of a premix with minerals and vitamins. The nutrient contents of the 4 diets hardly differed and met the requirements. There was no addition of Xylanase (X) and M in control diet which was offered to group I, while the diet of treatment II contained only X at common level. The birds of the treatments III and IV were fed diets with X like treatment II, additionally the usual (III) and the double of usual (IV) doses of M. The used NSP-degrading enzymes were endo-1,4-β-Mannanase and -Xylanase. Feed and water intake were measured daily and the body weight of each animal was determined weekly. At necropsy (days 22 and 37 of life, in total 32 birds per group) small intestine content was collected for the measurement of the viscosity (viscosimeter: Brookfield Ametek®, Middleborro, MA, USA). Furthermore a part of the distal ileum of 10 birds per group at day 37 of life was obtained, fixed in formol and subsequently stained (haematoxylin and eosin, alcian blue). Goblet cells were counted in 5 crypts and crypt depth as well as villus height were measured at 5 different localisations.

Statistical analyses were done by using the SAS® software (Cary, NC, USA) and included the Fisher's test for normally distributed dates. Not normally distributed data were evaluated with the Kruskal-Wallis test, more over pair-by-pair-comparisons were done with Wilcoxon signed-rank test.

Results: The doubling of M (IV) resulted in significantly higher mean final body weight. Furthermore in broilers fed diets including X a significant decrease in viscosity of the small intestinal content was observed, but there wasn't a further effect by adding M.

Treatment	I	II	III[1)]	IV[2)]
Body weight, d 36 (g)[3)]	$2528^{ab} \pm 291$	$2539^{ab} \pm 543$	$2500^{a} \pm 335$	$2642^{b} \pm 298$
FCR, d 1 - 36 (g/g)	1.62 ± 0.015	1.58 ± 0.032	1.59 ± 0.002	1.59 ± 0.017
Viscosity (mPa/s)[4)]	$3.77^{a} \pm 1.52$	$2.63^{b} \pm 0.268$	$2.64^{b} \pm 0.478$	$2.56^{b} \pm 0.332$
Goblet cells/crypt[5)] (n)	$40.0^{ab} \pm 12.9$	$38.5^{a} \pm 11.9$	$40.4^{b} \pm 12.8$	$40.1^{ab} \pm 14.1$
Crypt depth (µm)[5)]	$196^{a} \pm 79.8$	$179^{b} \pm 62.2$	$172^{bc} \pm 58.8$	$167^{c} \pm 57.7$
Villus height (µm)[5)]	$1614^{a} \pm 281$	$1596^{ab} \pm 335$	$1489^{b} \pm 287$	$1651^{a} \pm 314$

[1)]Enzyme activity of M 0.087 and [2)]0.176 (MU/kg), determined by LDG, Barcelona, Spain; [3)]$n_{group\ I/II/III/IV}$=51/51/49/51; [4)]n=31/group; [5)]n=10/group; [a, b, c, d]indicate significant differences (p

The depth of the jejunal crypt was lower in the groups fed diets including X, too. Contrary to expectations the heigths of the jejunal villi were not associated with the addition of M to the diet.

Conclusion: Digesta viscosity was only affected by adding X, not by including M in diets. Regarding the both added types of enzymes there were no effects on numbers of goblet cells and heigths of the jejunal villi, while the measurements of the crypt depth seemed to hinge on adding X. To sum up the parameters evaluated showed no effect of the M addition to a broiler diet including 30% SBM.

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79.

The effect of increasing dietary vitamin D_3 levels on growth performance and tibia bone measurements of chicks from four genetically diverse purebred layer lines during 12 weeks of rearing

Die Auswirkung steigender Vitamin D_3-Konzentrationen im Futter auf die Wachstumsleistung und Knochenmaße der Tibia von Küken vier genetisch divergenter Reinzuchtlegelinien während einer 12-wöchigen Aufzucht

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Vitamin D_3 represents a hormone-like substance that plays an important role in avian bone mineral metabolism and skeletal development through the regulation of the calcium- and phosphorus-homeostasis in blood. Because chicken's genetic origin and sex are strong influencing factors on growth performance and skeletal development, the present study investigated the effect of increasing dietary vitamin D_3 levels on growth performance and tibia bone measurements of male and female chicks from four layer lines differing in phylogeny and performance (WLA/R11: high/low performing white layers; BLA/L68: high/low performing brown layers) during 12 weeks of rearing.

Methods: After hatch 24 one-day-old chickens (3 males/3 females per line) were weighed and slaughtered for the dissection of both tibias. Additional 27 male and 27 female one-day-old chickens of each line were housed in six floor-range pens with one pen per sex and diet, providing 300 (acc. to GfE guidelines), 1000 (for grading) and 3000 (max. content acc. to EU feed additives regulation no 1831/2003) IE of vitamin D_3 (Cholecalciferol)/kg supply under *ad libitum* feeding conditions. From hatch to week 12 residual feed was recorded weekly per pen. Daily feed intake (DFI), daily weight gain (DWG) and feed to gain-ratio (FGR) were calculated. From every pen 3 chicks were weighed and slaughtered for the dissection of both tibias in four-week-intervals. The right tibia bone was analysed for its calcium and phosphorus concentration in accordance to the VDLUFA methods. Determination of dry bone weight and bone breaking strength (Model 4301, Instron, High Wycombe, United Kingdom) was performed on the left tibia. The statistical evaluation of data was performed with a four-factorial ANOVA (line, diet, sex and age - 4/8/12wk) by using Tukey-Kramer test in SAS procedure GLM ($p<0.05$).

			Week 4			Week 8			Week 12			
	Line	Sex	300 IE	1000 IE	3000 IE	300 IE	1000 IE	3000 IE	300 IE	1000 IE	3000 IE	PSEM
Relative bone weight (rBoW) (g/100g body weight)	WLA	m	0.245	0.266	0.267	0.296	0.271	0.279	0.298	0.319	0.319	0.014
		f	0.257	0.253	0.260	0.272	0.279	0.264	0.284	0.305	0.303	
	BLA	m	0.265	0.313	0.290	0.364	0.385	0.355	0.373	0.407	0.369	
		f	0.270	0.284	0.267	0.307	0.320	0.318	0.334	0.326	0.337	
	R11	m	0.291	0.294	0.281	0.334	0.278	0.329	0.335	0.325	0.323	
		f	0.295	0.287	0.278	0.335	0.300	0.296	0.314	0.329	0.325	
	L68	m	0.335	0.312	0.332	0.366	0.390	0.375	0.407	0.424	0.399	
		f	0.307	0.340	0.312	0.341	0.323	0.354	0.379	0.393	0.362	
Breaking strength (BS) (Newton)	WLA	m	63.3	73.7	59.3	122.3	116.0	133.7	148.7	149.7	165.7	10.6
		f	56.0	53.3	64.0	92.3	104.0	84.0	110.0	131.0	122.0	
	BLA	m	38.0	77.3	64.7	113.7	127.7	112.7	155.7	172.7	151.7	
		f	57.7	55.7	48.7	84.0	81.3	82.7	126.0	122.7	111.7	
	R11	m	51.3	47.3	64.7	85.0	87.3	87.7	148.3	111.7	134.3	
		f	49.7	51.7	43.7	67.7	61.7	69.7	100.3	102.3	100.3	
	L68	m	86.3	79.0	86.3	132.3	144.0	135.3	179.0	212.0	199.7	
		f	76.0	87.7	86.0	109.3	119.3	117.0	154.7	142.7	151.0	
Relative Calcium content (g/100g body weight)	WLA	m	0.044	0.048	0.050	0.056	0.054	0.053	0.063	0.068	0.064	0.002
		f	0.047	0.047	0.048	0.052	0.057	0.051	0.060	0.064	0.059	
	BLA	m	0.049	0.058	0.052	0.064	0.068	0.064	0.071	0.074	0.067	
		f	0.048	0.050	0.045	0.056	0.059	0.060	0.065	0.065	0.062	
	R11	m	0.053	0.051	0.052	0.062	0.051	0.059	0.069	0.063	0.064	
		f	0.051	0.053	0.053	0.060	0.056	0.057	0.067	0.067	0.066	
	L68	m	0.059	0.060	0.061	0.070	0.072	0.071	0.086	0.081	0.080	
		f	0.055	0.064	0.059	0.067	0.063	0.066	0.077	0.074	0.071	

ANOVA (p values)

	Genotype (GT)	Age(A)	Sex (S)	Diet (D)	GT*A	GT*S	GT*D	A*S	A*D	S*D	GT*A*S	GT*A*D	GT*S*D	A*S*D	GT*A*S*D
rBoW	<0.001	<0.001	<0.001	0.347	<0.001	<0.001	0.077	0.022	0.200	0.938	0.622	0.563	0.477	0.883	0.208
BS	<0.001	<0.001	<0.001	0.358	0.036	0.721	0.370	0.001	0.990	0.595	0.770	0.968	0.349	0.994	0.267
relative Ca content	<0.001	<0.001	<0.001	0.138	0.005	<0.001	0.006	0.098	0.008	0.711	0.343	0.451	0.287	0.967	0.549

Results: There were no significant effects of increasing dietary vitamin D3 levels on growth performance and bone measurements during the first 12 weeks of age (Table). FGR increased with aging ($p<0.001$), significant differences between the four genotypes were found only during the first 8 weeks of age. From 4 weeks of age, males had a lower FGR than females ($p<0.001$).

Table: Relative bone weight, breaking strength and relative Ca content of tibia bones

During the entire trial the calcium/phosphorus-ratio was always about 2.0 in each layer line and sex ($p\geq0.05$).

Conclusions: Performance and tibia bone measurements are primarily age-dependent. Sex differences occur from 4-8 weeks of age. In comparison to white layer lines, bones of brown layer lines, especially L68, are more stable with a higher concentration of calcium and phosphorus. During the investigation period the different dietary vitamin D_3 levels had no effect on performance and tibia bone measurements of the four genetically diverse purebred layer lines.

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80.

Dietary myo-inositol enhances serotonin and dopamine concentrations in plasma of 21-day-old broilers

Diätetisches Myo-Inositol erhöht die Plasmakonzentration von Serotonin und Dopamin in 21 Tage alten Broilern

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Question: The rapid growth of broiler chicken has to be balanced by an adequate dietary regimen. Not only macronutrients, but also minerals, trace elements and bioactive molecules are necessary to provide a nutritional supply according to the requirements. Bioactive myo-inositol, derived from endogenous synthesis or as dietary supplement is known to have manifold beneficial effects on metabolic processes in humans. In this context, the aim of this metabolomics study was to examine how dietary myo-inositol affects the plasma metabolic profile of young growing broilers.

Methods: Broiler hatchlings of the strain Ross 308 were placed in floor pens (stocking density: 11 animals per 2.6 m^2 pen) and were allocated to one of the two dietary treatments that only differed in myo-inositol supplementation. The MI group (n=8) was fed with 4.0 g myo-inositol/kg basal diet until day 21; the Control group (n=8) was fed with basal diet only. The diets were based on wheat, soybean meal and corn, and were offered for ad libitum consumption. During slaughtering at the age of 21 days, plasma samples were collected and were used to determine myo-inositol concentrations in Control and MI groups. Furthermore, a targeted and quantitative metabolomics analysis was performed in each individual animal using the Absolute IDQ p180 Kit of Biocrates (Innsbruck, Austria). Processed metabolomics data were evaluated by heatmap visualization and multivariate data analysis techniques such as principal component analysis (PCA) and partial least squares - discriminant analysis (PLS-DA).

Results: Myo-inositol supplementation resulted in significantly higher myo-inositol plasma concentrations: 50.6±3.7 mg/l vs. 100.4±11.6 mg/l (mean±SEM, P=0.0008) in the Control vs. MI group, respectively. The metabolomics analysis resulted in the quantitative identification of 185 plasma metabolites, belonging to the following metabolite classes: acyl-carnitines, amino acids, biogenic amines, phosphatidylcholines (PC), lyso-phosphatidylcholines (lyso-PC), sphingomyelines (SM) and hexoses. The bioinformatic analysis of the metabolomics data revealed as major finding that dietary myo-inositol in broiler chicken enhanced serotonin and dopamine concentrations significantly (Control vs. MI: serotonin 12.01±4.26 vs. 33.91±8.30, P=0.03, dopamine: 0.25±0.02 vs. 0.43±0.07, P=0.03, all in µmol/l, mean±SEM). Average daily gain and feed intake were not affected by the dietary treatment. Further details of zootechnical parameters are presented elsewhere (Sommerfeld et al., GfE Conference 2017).

Conclusions: Both serotonin and dopamine are endocrine signals involved in positive modulation of behavior, feed intake regulation, glucose-insulin homeostasis and animal welfare. These findings can be used to create new research hypotheses on the beneficial biological role of myo-inositol. Furthermore, complete results of the metabolomics analysis as a systemic holistic approach can be used to get a deeper insight into metabolic regulation and inflammatory responses in chicken.

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81.

Effect of periparturient sows antioxidant supplementation on piglets survival at birth and sows feed intake during lactation

Effekt einer peripartalen Antioxidantien-Supplementation auf das Überleben der Ferkel bei der Geburt und die Futteraufnahme der Sauen während der Laktation

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Pregnancy and farrowing are periods of oxidative stress, not only for the sow but also for the newborn piglet (1). Oxidative stress is high at the beginning of gestation, moderate at the moment of histological differentiation of fetal organs and tissues, and then increases progressively towards the end of gestation. An adequate supplementation of antioxidants is therefore of interest for maximizing reproductive performances and improving vitality, development and growth of newborn piglets (2). A previous study already showed a beneficial effect of delivering antioxidants to the sow during weaning-estrus interval on the percentage of mature piglets per litter and on the within-litter variation of birth weights (3). The purpose of the present study was to assess the effect of antioxidants supplementation (SOD and organic selenium, acting as a cofactor of glutathione peroxidase) on sows reproductive performance and piglets survival at birth.

Methods: The tested antioxidants were organic selenium, acting as a cofactor of glutathione peroxidase (Alkosel®: 1 g/sow/day) and superoxide dismutase (SOD)-rich melon pulp concentrate (MPC) (Melofeed®: 0.3 g/sow/day), given in top-feeding to the sow from 8 days before farrowing until piglets weaning at 21 days. The field trial was performed on 169 sows: 63 sows were supplemented with organic selenium (A), 54 sows were supplemented with a combination of organic selenium and SOD-rich MPC (A+M) and 52 sows were not supplemented (control group: C). The percentage of stillborn piglets at birth and the percentage of sows having 2 or more stillborn piglets were calculated. To take into account the litter size effect, the percentage of stillborn piglets in the trial was also compared to the equation established from several farms: *% of stillborn = 0.87 × litter size - 4.04* (Lallemand, internal data, n = 66 sows). The data were analyzed by a linear mixed model with SPSS Statistics 21, according to the supplemented group (C, A, A+M) and the sow's parity (from 1 to 5, with parity 1 being analyzed separately).

Results: The percentage of stillborn piglets was decreased by half in the group A+M (4.5%) compared to the group C (7.9%) ($p = 0.064$) for sows in parities 2-5, the group A being intermediate (6.2%). This reduction was also observed for gilts (C: 7.7%, A: 4.8%, A+M: 4.9%). In addition, the combination A+M decreased the percentage of sows having 2 stillborn piglets or more (16%), compared to group C (35%) and the group A had intermediate values between groups C and A+M (23%). The combination A+M also increased the percentage of sows having less stillborn piglets than the expected value given by the equation: *% of stillborn = 0.87× litter size - 4.04*.

Moreover, sows in parities 2-5 in A and A+M groups had significantly higher feed intake during lactation than control group (6 kg/sow/day). Organic selenium increased feed intake by 540 g/sow/day over control group. Organic selenium + SOD increased feed intake by 580 g/sow/day over control group. This effect may be explained by less stress and a lower impact of stress on feed intake.

Conclusions: The antioxidant supplementation with the combination selenium yeast (Alkosel®) and SOD (Melofeed®) proved to be beneficial and synergistic to decrease piglets mortality rate, analyzed by different criteria (percentage of sows having only 0 or 1 stillborn piglet and comparison to expected values). Moreover supplementation of this antioxidant combination to the sows from 8 days before farrowing until weaning improved feed intake of sows during lactation. Higher feed intake during lactation can induce more milk produced and/or less loss of body condition, therefore better reproduction conditions should be expected at the next parity.

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82.

The inflammatory response of porcine monocyte-derived dendritic cells to treatment with probiotic *E. faecium* and pathogenic *E. coli* strains

Die inflammatorische Antwort porciner Blutmonozyten-abgeleiteter dendritischer Zellen auf die Behandlung mit probiotischen E. faecium- und pathogenen E. coli-Stämmen

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In the gut, there is a mutual interaction between microbiota, epithelium and immune cells influencing the inflammatory state of the intestinal mucosa. Pathogens can either bind to extracellular receptors at the cell membrane (Toll-like receptors) or intracellular receptors of the NLR (nucleotide-binding domain und leucine-rich repeat containing) protein family (1). The NLRP3 inflammasome is activated by a two-step process, which contains a first priming step and a second step causing the formation of a functional multiprotein complex. This in turn leads to secretion of pro-inflammatory cytokines.

Probiotic bacteria have been shown to activate the inflammasome (2). The probiotic *Enterococcus faecium* NCIMB 10415 (*E. faecium*) has previously been demonstrated to influence immunological functions and to reduce the incidence of *Escherichia coli* (*E. coli*)-induced diarrhea (3). We hypothesized that the NLRP3 inflammasome is involved in those probiotic effects. The aim of the study was to monitor the response of dendritic cells towards probiotic and enteropathogenic bacteria in the gut.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood by density gradient centrifugation. Thereafter monocytes were isolated by magnetic cell sorting using CD14 MicroBeads. $CD14^+$ cells were plated in 24-well plates and differentiated into immature monocyte-derived dendritic cells (MoDCs) within 6 d in the presence of recombinant porcine (rp) granulocyte-macrophage colony stimulating factor (GM-CSF) and rp interleukin-4 (IL-4). Flow cytometric analysis for myeloid specific cell surface markers were performed on days 0 and 6. For activation of the NLRP3 inflammasome, lipopolysaccharide (LPS) was used as a priming signal. After 3 h, MoDCs were incubated for 1-2 h with *E. faecium*, enterotoxigenic *E. coli* (ETEC), and typical stimulants for the inflammasome. mRNA expression of inflammasome components was analyzed by quantitative real-time PCR. At the protein level, pro-inflammatory cytokines were measured in cell culture supernatants *via* ELISA. Furthermore, NLRP3 protein expression was assessed by Western blot. Statistical evaluation of the data was performed, if applicable, by variance analysis and a post-hoc LSD test.

Results: Flow cytometric analyses revealed that $CD14^+$ monocytes were consistently and purely isolated by magnetic cell separation. Following DC differentiation, adherent cells were shown to be positive for CD14, CD16, CD1 and swine leucocyte antigen (SLA) II. NLRP3 mRNA expression increased 2.6-fold after priming the cells with LPS ($p < 0.05$). This effect was not further augmented by bacterial incubation at the mRNA level. This corresponds to the activation of transcription during the first priming step. Similarly, mRNA expression of the cytokines IL-1β and IL-18 was upregulated 73.3-fold and 1.9-fold, respectively, by LPS priming ($p < 0.05$). Preliminary data indicate a higher release of pro-inflammatory cytokine IL-1β in LPS primed and *E. faecium*- and ETEC-incubated cells compared to untreated cells (3.3-fold in LPS primed cells as well as in *E. faecium*-incubated cells, and 12.3-fold in ETEC-incubated cells, respectively). In addition, a 9.3-fold upregulation of this cytokine by adenosine triphosphate (ATP), which served as a positive control stimulant, could be observed.

Conclusion: These results indicate that MoDCs are able to respond to bacteria occurring in the gut and we found that components of the inflammasome are activated. Hence, MoDCs could be useful for co-culture studies with intestinal epithelial cells when investigating probiotic mechanisms.

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83.

Ex vivo studies on survival of *Salmonella Derby* exposed to porcine gastric contents with/without Benzoic acid

Ex vivo - Untersuchungen zur Überlebensrate von Salmonella Derby im Mageninhalt von Schweinen unter dem Einfluss eines Benzoesäure-Zusatzes

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The presence of *Salmonella* spp. in pork production is still a challenge for human health. To reduce the prevalence of this zoonotic bacterium in pork production, efforts should be focused on prevention rather than treatment [1]. Gastric pH-value is a well-known barrier for pathogens [2,3]. Therefore, acidifying feed additives (e.g. organic acids) are frequently used. Benzoic acid is a licensed feed additive, which apart from further effects reduces urinary pH in sows. It was hypothesized that the addition of Benzoic acid to porcine stomach contents influences the survival rate of Salmonella.

Material and Methods: The gastric contents of 8 pigs (BHZP Hybrid Pietrain ♂ x Victoria ♀, 3-4 months old) fed conventional wheat based fattening diets (DM: 884 g/kg, 14.8 MJ ME/kg DM, CP: 188 g/kg DM, CF: 50.1 g/kg DM) were collected, thoroughly homogenized and divided into two aliquots (A and B). Each aliquot was subsequently divided equally into five plastic bags (A1-5 and B1-5). Intended as feed additive 1% of Benzoic acid was added to the gastric content in each plastic bag of aliquot B. All five parts of aliquot A and B were inoculated with *Salmonella* Derby (~6.5*10^7 colony forming units per gramm, CFU/g) simultaneously and placed in a water bath for incubation at 37 °C. After 0, 30, 60, 120 and 240 minutes of storage, one plastic bag of aliquot A and B was removed from the water bath. The pH-value of gastric content was measured followed by serial dilution and microbiological analysis of Salmonella (CFU/g). Data in the table below were analysed with t-test (normally distributed) and with Wilcoxon-test (not normally distributed).

Results: The pH-values of gastric contents of aliquot B (including Benzoic acid) were on average lowered by 0.22 pH-units compared to aliquot A at every point of time (Table).

incubation [min]		0	30	60	120	240
	n (A/B)	8/8	8/8	8/8	8/8	8/8
mean pH, gastric contents	without acid (A)	4.38[a] ±0.33	4.38[a] ±0.35	4.46[a] ±0.38	4.41[a] ±0.34	4.41[a] ±0.32
	with acid (B)	4.19[a] ±0.28	4.18[a] ±0.27	4.18[a] ±0.26	4.20[a] ±0.27	4.21[a] ±0.26
mean CFU Salmonella (log/g gastric content)	without acid (A)	7.77[a] ±0.18	7.48[a] ±0.35	7.08[a] ±0.92	6.64[a] ±1.51	6.10[a] ±2.03
	with acid (B)	7.73[a] ±0.15	6.19[a] ±1.35	6.04[a] ±1.17	4.30[b] ±2.69	3.62[b] ±2.79

Different superscripts ([a], [b]) indicate significant differences at the same point of time ($p<0.05$)
Furthermore, the counts (CFU) of surviving Salmonella/g gastric content during 240 min of incubation were markedly lower in aliquot B (with Benzoic acid).

Conclusion: Results indicate that supplementation of Benzoic acid at 1% might act as an additive improving gastric barrier function. It lowers the pH-value of gastric content consistently however the differences were not statistically significant. Its effect on reducing Salmonella units was observed despite its poor water solubility, which could not be altered even at low pH-conditions. At 120 and 240 min of incubation the differences on Salmonella counts achieved statistical significance. Due to high standard deviations, further investigations on the Salmonella survival rate in the gastric contents with/without Benzoic acid are needed.

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84.

Effects of different Zinc formulations in 25 - 39 days old piglets

Wirksamkeit unterschiedlicher Zink-Formulierungen bei Ferkeln vom 25. bis 39. Lebenstag

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The aim of the present study was to investigate the potential of zinc in different formulations in piglets during a 7-day zinc repletion period (70 ppm) after a previous 7-day depletion phase (40 ppm zinc) compared to piglets without appropriate supplement.

Methods: The piglets selected for the experiment were evenly divided into 6 groups without and with addition of zinc (30 ppm) in different forms (zinc as: oxide, sulfate, glycinates, methionate, protected oxide (MiaTrace^{R}) to a basal diet with the main components wheat, barley, soya meal, corn, whey powder. For each experimental group 5 repetitions with 10 animals each were used. Performance, including feed : gain ration, health status and faeces consistency were measured. At the end of the 7-day zinc repletion period (39th day of life), the apparent ileal digestibility of zinc was determined according to the indicator method with titanium (IV) dioxide as indicator. For the assessment of zinc status, blood was sampled from the vena cava cranialis at the end of the 7-day zinc repletion period, blood counts, the activity of the alkaline phosphatase and the serum zinc contents were measured. Liver and kidneys were analyzed the zinc contents, in addition, the expression of metallothionein was determined in the liver by means of quantitative real-time PCR. The statistical analysis was carried out using SPSS (IBM SPSS, Statistics 21). After checking the homogeneity of the variances, ANOVA and Tukey tests were performed. The significance level was set to $P < 0.05$. The expression of the metallothionein genes MT1a and MT2a was determined by the software REST 2009.

Results: With almost identical initial weights at the beginning of the 7-day repletion period, all piglets were clinical healthy and faeces quality was comparable. The piglets fed the zinc supplement diets increased their weight by 28.5%, differences compared to the control group were, however, only significant with the addition of zinc glycinate, methionate and MiaTrace^{R}. The apparent ileal digestibility of zinc was significantly improved by 23.4% in the supplemented groups compared to the control group. The organic zinc formulations tended to have an 8.4% higher apparent ileal zinc digestibility compared to the inorganic zinc salts. The differences could be statistically confirmed between zinc oxide against glycinate and MiaTrace^{R}. The blood counts were similar between the groups, the zinc contents analyzed at the end of the 7-day repletion period in the blood serum increased, however, the data could only be statistically confirmed after the addition of zinc-glycinate. The concentration ranges for zinc methionate and MiaTrace^{R} were higher (+ 13.6%) compared with inorganic zinc formulations (n.s.). The activity of zinc-dependent alkaline phosphatase was higher compared to the control group for the group fed the diet with MiaTrace ($p<0.05$). The zinc concentration in liver and kidney in the control group were 103 and 56 mg/kg, respectively. The piglets fed with different zinc formulations showed an average increase of 15.8% (liver) and 23.4% (kidney) compared to the control group. However, due to the individual variability, the differences compared to the control group were not significant. The expression of the metallothionein isomers MT1a and MT2a in the liver of the test groups supplemented with zinc showed a MT1a expression increased by 6.85 to 11.41 fold compared to the depleted control animals. The numerically highest increase in MT1a expression was obtained with the addition of zinc methionate. In contrast, the expression of the metallothionein isomer MT2a showed a clear dependence on the selected zinc formulation. It was found that the organic forms of zinc-glycinate and methionate and MiaTrace^{R} resulted in a lower increase in relative gene expression compared to zinc oxide and zinc sulfate.

Conclusion: The tested zinc formulations were effective to increase the zinc status of young piglets after a short term depletion period. The data indicate different efficacy and impact on zinc indicators including the gene expression of metallothionein.

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85.

Decontamination of deoxynivalenol (DON) contaminated maize with Na_2SO_3: Treatment effects on health of piglets in a LPS-challenge model

Dekontamination von Deoxynivalenol (DON) kontaminiertem Mais mit Na_2SO_3: Behandlungseffekte auf die Gesundheit von Ferkeln in einem LPS-Challenge-Modell

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Treatment of DON-contaminated feedstuffs with sodium sulfite (Na_2SO_3, SoS) reduced DON content markedly and counteracted the adverse DON-effects in pigs (1). However, some of these data pointed at interactive and unspecific SoS effects on health of piglets. Therefore, we specifically addressed these interactions by using a lipopolysaccharide (LPS) challenge model. Such a model enables studying treatment effects under inflammatory stress conditions.

Methods: 80 piglets (7.57±0.92 kg BW) were equally divided into four groups based on following diets: CON- (control diet with 10% maize), CON+ (diet with 10% maize wet-preserved with 5g SoS/kg), FUS- (diet with 10% mycotoxin-contaminated maize with 5.36mg DON/kg), and FUS+ (diet with 10% mycotoxin-contaminated maize, wet-preserved with 5g SoS/kg; 0.8 mg DON/kg). After 42 days, half of each group (n = 10) was injected intraperitoneally either with 7.5 µg LPS/kg BW (LPS: *E.coli* O111:B4) or with 0.9% NaCl/kg BW (volume ~ 6.5 mL). Over a period from -15 min before to 120 min after injection, clinical signs were recorded every 30 min and based on this a cumulative clinical score calculated. At 120 min, blood samples were collected to analyse haematological profile, clinical biochemistry, nitric oxide (NO) production and FRAP (ferric reducing ability of plasma). Data were statistically analysed with a 3-factorial ANOVA (feed: CON vs. FUS, SoS: with or without, LPS: injection of NaCl or LPS) and differences (Student's *t*-test) were considered significant at $p \leq 0.05$.

Results: LPS stimulated pigs clearly showed a higher cumulative clinical score with prominently injected episcleral vessels. Besides, a significantly LPS induced increase of body temperature (by ~ 0.5°C) was observed (Fig 1). Two hours after injection, LPS also significantly increased hemoglobin ($p_{LPS} = 0.027$), but caused no other changes in red haemogram. In general, LPS provoked a leukopenia in all groups (p_{LPS}FUSxSoSxLPS = 0.033). Furthermore, LPS significantly increased γ-glutamyltransferase (γ-GT) and bilirubin concentrations. Besides, a significant interaction between LPS and FUS on aspartate aminotransferase (AST) was observed ($p_{FUSxLPS} = 0.028$): AST was elevated by LPS with the exception of group FUS+/LPS where AST levels were lower. Investigation of the animal's redox state showed no alteration of NO production. FRAP was strongly increased in FUS-/NaCl group compared to their CON-/NaCl counterparts, whereas LPS presence decreased FRAP in FUS- fed pigs, but increased its values in CON-fed pigs. Moreover, diets treated with SoS did not [illegible] injected animals ($p_{FUSxSoSxLPS} = 0.021$).

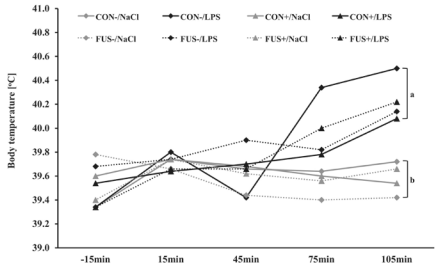

Figure 1 Effect of DON, SoS and LPS challenge on the body temperature (in 30 min intervals). Data are presented as LSMeans and statistical major effects were distributed as follows: $p_{FUS} = 0.76$; $p_{SoS} = 0.69$; $p_{LPS} = 0.02$; $p_{time} < 0.001$; $p_{DONxSoSxLPSxtime} < 0.001$. $^{a, b}$: significant difference ($p \leq 0.05$).

Conclusions: Our results indicate that chronic dietary DON exposure as well as SoS-treatment of diets had a significant impact on piglet's health parameters, but not always as a clear-cut picture. SoS elicited opposing effects on leukocyte counts dependent on DON-presence in diets and diminished the impact of DON and LPS on FRAP. Further analyses elucidating the observed effects are in progress.

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86.

The effect of replacing plasma with increased levels of phytase and soy protein concentrate in a piglet diet

Der Einfluss von erhoehtem Einsatz von Phytase und Soja-Protein-Konzentrat anstelle von Plasma in einer Ferkelration

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Where the use of plasma in piglet feeds is acceptable from a regulatory and consumer point of view this material is routinely used to improve performance during the first 14 days after weaning. Whilst the cost of including plasma is high it is normally justified in terms of performance achieved. The application of new generation phytases at high doses - superdosing - to eliminate the anti-nutrient effects of phytate rather than simply improve mineral supply, has been shown to improve piglet performance (Cordero et al, 2013). The current trials were designed to determine if the replacement of plasma with phytase superdosing can achieve similar performance, whilst reducing feed cost.

Methods: A 3% plasma/5.5% soy protein concentrate containing piglet starter diet (Plasma) with 500 FTU/kg phytase (Quantum® Blue, AB Vista) was compared to a diet without plasma where the soy protein concentrate was increased to 9% and the phytase inclusion was increased to 2000 FTU/kg (SD) in two successive piglet trials performed in Spain. The diets were formulated to be iso-nutrient including digestible P and calcium (0.40% and 0.9%, respectively) with no matrix attributed to the inclusion of phytase above 500 FTU/kg, and were fed ad libitum from 1-14 days post weaning. Each trial had 8 replicate pens per treatment and 38 piglets per pen (mixed sex). Pigs were weaned at 21 days, with start weights in trial 1 of 6.09 ± 0.01 kg and 6.02 ± 0.01 kg in trial 2. Data were analysed as a simple ANOVA, using diet as the factor.

Results: In trial 1 daily gain and FCR were improved by the use of the SD diet. In trial 2 only the FCR was improved by the use of the SD diet. Performance in trial 1 was much lower than in trial 2, indicating some level of challenge in this trial whilst the performance in trial 2 was at a normal level for the facility.

Table 1: Performance results 1-14 days post weaning

	Trial 1		Trial 2	
	PLASMA DIET	SD DIET	PLASMA DIET	SD DIET
Daily Gain (g/d)	98[a]	122[b]	143	154
FCR (g/g)	1.76[a]	1.44[b]	1.31[a]	1.19[b]

[a,b] If different within trial indicates significant differences (P

As the SD diet was around 100 euro/tonne cheaper than the PLASMA diet and FCR was improved for the SD diet the costs per piglet were reduced by 0.24 Euro in trial 1 and 0.31 Euro in trial 2.

Conclusion: The increased inclusion of phytase at 2000 FTU/kg without use of plasma resulted in equivalent or better performance than the plasma containing diet with standard level of phytase, showing that the negative effects of phytate can be negated by using higher levels of new generation phytase.

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87.

Impact of α-amylase supplementation on energy balance and performance of high-yielding dairy cows on moderate starch feeding

Einfluss einer Supplementierung mit α-Amylase auf die Energiebilanz und Leistung hochleistender Milchkühe bei moderater Stärkeversorgung

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Energetic deficiency is a common situation in high-yielding dairy cows during early lactation. It is associated with considerable lipomobilization and increased risk for poor fertility. Particularly primiparous cows (PRIC) are predisposed due to continuous growth. We hypothesized that supplemental α-amylase might improve energy utilization from starch even on moderate starch intake and thus support the adaptive response to energetic deficiency during early lactation. Therefore, we investigated effects of α-amylase supply on performance, health traits linked to energy balance and fertility in PRIC and pluriparous cows (PLUC) from a commercial herd over a period of one year.

Methods: 421 PRIC and PLUC received a total mixed ration *ad libitum* and up to 1 kg/d of a pelleted concentrate *via* transponder feeding. In the treatment group (TG), the concentrate contained α-amylase (Ronozyme® Rumistar™ 600 CT) to achieve 300 KNU (kilo novo units)/kg dry matter (DM) of the total diet, assuming a DM intake of 25 kg/d. Control group (CG) cows received the same concentrate without α-amylase additive. The daily concentrate intake (DCIT) was recorded automatically by the feeding-on-demand system. Statistical evaluation considered all cows included in the study irrespective of their actual intake (evaluation A, EVA), and furthermore exclusively cows realising a minimum intake of ≥ 0.8 kg DCIT, corresponding to 240 KNU/d in the TG (evaluation B, EVB). This threshold is presumed to reflect the minimal effective enzyme dosage. The individual daily milk yield (DMY) was recorded in the milking parlour. Milk protein (MP), urea (MU), fat (MF), lactose (LA) and somatic cell counts were determined once per month. Somatic cell score (SCS) and fat-to-protein ratio (FPR) in milk were calculated. Total bilirubin (TB) and β-hydroxybutyrate (BHBA) in blood serum and backfat thickness (BFT) were determined at parturition and on days in milk (DIM) 30, 45, 90, 120 and 180. Fertility was characterised by the first insemination success (FIS) and insemination index (II). Cows were further allocated depending on their stage of lactation into a high *vs* low lactation group (HLG *vs* LLG: ≥ *vs* < 32 kg milk/d), receiving different diets. Statistical analysis was performed using random (DMY, DCIT, MP, MF, LA, FPR and SCS) or fixed regression test-day models (MU). Least squares means were estimated for DIM. BFT was analysed using a fixed regression model. TB and BHBA were analysed considering fixed effects for combinations of experimental group, parity, time of measurement and fixed year-month effects. FIS and II were analysed using generalized linear models with fixed experimental group and parity effects. P values < 0.05 were considered significant. All traits were analysed using SAS 9.4.

Results: The TMR starch content was 220 ± 20.8 *vs* 183 ± 24.8 g/kg DM in HLG *vs* LLG. Mean DCIT was consistently below target, with a slightly higher intake in TG PRIC (0.9 ± 0.3 kg) than in TG PLUC (0.8 ± 0.3 kg). EVA and EVB identified different DMY of CG *vs* TG only in PRIC until 100 DIM (EVA: 30.8 ± 0.50 *vs* 32.2 ± 0.49 kg; EVB: 30.9 ± 0.62 *vs* 32.8 ± 0.55 kg; $P < 0.05$). MP, MU, MF and LA contents, FPR and SCS were unaffected by α-amylase supplementation ($P > 0.05$). There was no systematic effect of the treatment on BFT, TB, BHBA and fertility traits as well ($P > 0.05$). Neither did SCS indicate any increased risk for mastitis, nor did BFT, TB and BHBA indicate ketosis.

Conclusion: The results of this study suggest that PRIC seem to benefit from exogenous α-amylase as an additive in early lactation, even if starch supply is moderate. A resultant elevation of DMY has been shown. PLUC apparently do not benefit from α-amylase supplementation in a similar manner.

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88.

Effect of the flavanols catechin, gallocatechin, epigallocatechin and epigallocatechin gallate on *in vitro* ruminal fermentation

Einfluss der Flavanole Catechin, Gallocatechin, Epigallocatechin und Epigallocatechingallat auf die ruminale Fermentation in vitro

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Some phytogenic compounds such as polyphenols have been shown to have health promoting and methane (CH4) mitigating effects (1). Furthermore, polyphenols can influence ruminal fermentation and microbial population in the rumen (2). A number of pure flavonoids belonging to the group of polyphenols have been investigated before (3). For the present study, another four flavonoids from the subgroup of the flavanols, with known health promoting properties (e.g. antioxidant, anti-cancerogenic and anti-inflammatory) were selected. These were catechin, gallocatechin, epigallocatechin and epigallocatechin gallate. The aim of this study was to test whether and how the addition of these four flavanols to ryegrass hay affects the ruminal fermentation when given in purified form. We hypothesize that the mentioned flavanols do not affect ruminal fermentation in a negative way.

Methods: Short-term effects of the four flavanols on ruminal fermentation were examined with the Hohenheim gas test, an established *in vitro* system. Dosages of 0.1, 1.0 or 10 mg (equivalent to 3.3, 33 or 333 µg/ml incubation fluid) of catechin, gallocatechin, epigallocatechin or epigallocatechin gallate were incubated together with 200 mg dry matter of ryegrass hay. This concentration of polyphenols up to 5% in feed DM is common when supplementing feed with plant extracts rich in polyphenols. A non-supplemented control and tannic acid supplementation as a positive control (3) were included as well. The feeds were added to 30 ml ruminal fluid-buffer solution (1:3) for 24 h at 39 °C. All treatments were tested in four runs (n = 4). Ruminal fluid was collected before morning feeding from a rumen-fistulated dairy cow. After 24 h, the incubation fluid was analyzed for pH, ammonia concentration, short chain fatty acids (SCFA), as well as protozoal and bacterial counts. In addition, fermentation gas production and CH_4 concentration in fermentation gas was determined. Data was analyzed with the GLM procedure of SAS considering dietary treatment as fixed effect and run as random effect; this separately per dosage.

Results: The 10 mg dosage of tannic acid and epigallocatechin gallate decreased (P4 formation by 20.3% and 12.6%. Furthermore, tannic acid lowered (P4concentration in fermentation gas by 10.6% with the 10 mg dosage. Fermentation gas formation was lower with tannic acid, gallocatechin and epigallocatechin gallate (-11.6%, - 10.7% and -9.0%, respectively). The pH of the incubation fluid as well as bacterial and protozoal counts were not affected by flavanol or tannic acid supplementations. Supplementing 10 mg flavanol reduced (P

Conclusion: The results show that tannic acid, but also epigallocatechin gallate, have the potential to lower CH_4 mitigation. The reduced concentrations (mmol/l) of valerate and iso-valerate in the incubation medium may indicate an inhibition of the number of valerate / iso-valerate-forming microbes involved in fiber degradation. Long-term *in vitro* as well as *in vivo* studies are necessary to establish a comprehensive knowledge about these flavanols in ruminant nutrition, especially also concerning side-effects on fiber digestion, so that they can be strategically used to improve oxidative status and lower CH_4 mitigation by ruminants.

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89.

Effects of isoquinolene alkaloids on milk yield and somatic cell count in dairy cows

Einfluß von Isochinolin-Alkaloiden auf Milchleistung und somatische Zellzahlen bei Milchkühen

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Introduction: It has been demonstrated that isoquinoline alkaloids (IQ), secondary plant components extracted from *Macleaya cordata* (Papaveraceae), have anti-inflammatory and immunomodulatory effects in various species (1,2). Due to their possible positive effects in ruminants the main objective of this study was to determine the effect of supplementation with an IQ-containing plant extract (IQPE) on milk production and milk quality in German Holstein cows during the first 100 days of lactation.

Material and Methods: The experiment was conducted in a commercial farm in Thuringia with more than 600 milking cows. A pool of 93 cows were randomly divided into two groups: control group (CG, without additive) and experimental group (EG) supplemented with 8 g IQPE/*cow/d* (*Sangrovit® RS, Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany)* from 3 weeks ante partum to 15 weeks post partum. Both groups were fed the same total mixed ration (TMR) according to the recommendations of GfE (2009). Milk data were recorded (average milk recording time on 18th day p.p.; 50th day p.p. and 80th day p.p.) and additionally colostrum samples were taken to evaluate the IgG and total protein content. From the milk recording association of Thuringia (TVL) the 100-day-milk yield for all cows in the present investigation were calculated. For analysis the statistical software SPSS (21.0) and R (3.2.2; R Development Core Team 2015) were used for analysis of variance between both groups.

Results: The colostrum showed an average protein content of 167 ± 42 mg per ml, with no significant differences between the cows of both groups (CG: 165 ± 53 mg/ml and EG: 168 ± 42 mg/ml). Supplementation of IQPE showed a significant ($p < 0.05$) effect on milk yield and amount of milk protein during the first 3 milk recording times (table 1). Milk fat and amount of urea were only affected by milk recording time (MRT). Interactions between groups and milk recording time were not determined.

Table 1: average daily milk yield and composition of milk in both groups (n=93)

	CG	EG	SEM		p-value	
				group	MRT	group x MRT
milk yield (kg/d)	35.5	37.4	0.40	0.022	0.214	0.888
milk fat (g/d)	1,574	1,637	20.6	0.128	< 0.001	0.806
milk protein (g/d)	1,171	1,223	12.0	0.033	0.008	0.670
Somatic Cell Count (x 1000)	289	152	54.99	0.241	0.637	0.341
urea (mg/l)	256	257	3.7	0.974	< 0.001	0.595

100-day-milk yield of cows were 195 kg higher in experimental group than by cows of control group, but there were no significant differences (table 2).

Table 2: 100-day-milk yield of cows in both groups (n=93)

	milk yield (kg)	fat (kg)	protein (kg)
Control group	3,537 ± 554	156 ± 30	117 ± 16
Experimental group	3,732 ± 539	163 ± 26	122 ± 16

Conclusion: The present study indicates that supplementation of isoquinoline alkaloids from a standardized plant extract during the transit period leads to a significant increase of milk yield and milk protein in dairy cows during first 100 days of lactation.

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90.

Effects of a dietary niacin supplementation on muscle fibre composition and related metabolism in dairy cows

Effekte einer Niacinsupplementierung auf die Muskelfaserzusammensetzung und den damit verbundenen Stoffwechsel von Milchkühen

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Dietary supplementation of niacin has been shown to cause fibre-type switching in the skeletal muscle towards oxidative type 1 fibres, and results in a muscle metabolic phenotype favouring fatty acid (FA) utilization (1). This may be helpful in situations with high need for FA oxidation. In high-yielding dairy cows which experience a negative energy balance, lipolysis accompanied by high availability of non-esterified FA (NEFA) can overstrain liver capacity for FA oxidation, and favour triglyceride (TG) synthesis and ketogenesis. Therefore, we tested the hypothesis that dietary supplementation of niacin to transition cows increases type 1 fibres and FA oxidation in the skeletal muscle and reduces ketogenesis in the liver.

Methods: Thirty multiparous Holstein-Friesian cows (682±60 kg body weight) were allocated to 2 groups and fed either a grass/maize silage based total mixed ration (TMR) (forage: concentrate 60:40) (control group) or the same TMR plus ~55 g/cow/day of a rumen-protected niacin product (Niashure; Balchem, New Hampton, NY, USA) (45 mg duodenal available niacin/kg body weight) (niacin group) from 3 wks a.p. until 3 wks p.p. Feed intake and milk yield were recorded daily. Blood samples were collected weekly, and biopsies of liver, M. *longissimus lumborum* between 4th and 5th lumbar vertebra (LD) and M. *semitendinosus* ~15 cm ventral of *Tuber ischiadicum* (ST) 3 wks a.p. and 1 and 3 wks p.p. Plasma concentrations of nicotinic acid and its metabolite nicotinamide (NAM) (HPLC-MS/MS), NEFA and β-hydroxybutyrate (BHB), and liver TG, were determined. The expression of genes involved in FA utilization, ketogenesis, and muscle fibre composition was determined in liver and/or muscle by real-time PCR and muscle fibre composition by immunohistochemistry. The data were analysed by analysis of variance with treatment, time and their interaction as fixed and animal as random effect.

Results: At 3 wks a.p., plasma NAM concentrations were similar in both groups (P=0.13), but were higher p.p. in niacin-supplemented cows (2.5±0.5 µg/ml) than in control cows (0.6±0.2) (P<0.001). P.p. dry matter intake (control 16.0±3.4, niacin 15.6±2.4 kg) and milk yield (control 38.1±6.0, niacin 35.8±6.7 kg) were similar in both groups (P>0.1). However, milk fat yield was lower in the niacin (1.8±0.5 kg/d) than in the control group (2.1±0.5) (P=0.035). In the LD and ST muscle, transcript abundance of genes involved in muscle fibre-type switching, FA uptake, the carnitine shuttle, ß-oxidation and oxidative phosphorylation were largely similar between treatment groups. Likewise, the fibre type composition was largely similar between groups in the LD muscle (1:2a:2x = 32±5:34±6:34±8; p.p. mean±SD) and the ST muscle (1:2a:2x = 22±5:34±7:44±8). Liver TG (~70-80 mg/g liver; 1 wk p.p.) and plasma NEFA concentrations increased with the onset of lactation in both groups. However, 3 wk p.p., plasma NEFA concentrations were lower in the niacin (0.40±0.22 mM) than in the control group (0.58±0.25) (P=0.045). In the liver, mRNA abundance of FGF21, a regulator of ketogenesis, increased about 18-fold with the onset of lactation; in addition, it was lower in the niacin group compared to the control group in week 3 p.p. (P=0.002) which indicates that liver ketone body synthesis may have been decreased. Correspondingly, 2 wk p.p., plasma BHB concentrations were in tendency lower in the niacin (0.62±0.22 mM) than in the control group (0.77±0.20) (P=0.069); however, they were below the threshold for subclinical ketosis (1.2 mM) in both groups. The expression of genes involved in the carnitine shuttle, in ß-oxidation of FA and in TG synthesis was not influenced in the liver (P>0.1).

Conclusion: We show that high doses of rumen-protected niacin (2 to 5-fold above those generally used in dairy cows) partially reduced plasma NEFA and BHB concentrations in transition cows. However, the hypothesis that changes of the muscle metabolic phenotype may underlie these positive effects could not be verified.

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91.

Glycemic and insulinemic response of warm-blooded mares after feeding of Jerusalem artichoke meal

Glykämische und insulinämische Reaktion von Warmblutpferden auf die Fütterung von Topinamburmehl

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Dietary supplementation with fructans may increase insulin sensitivity and reduce insulinaemic response following intake of soluble carbohydrates in obese horses without affecting body weight and body condition score (1). We hypothesized that even metabolic and clinically healthy, non-obese horses might benefit from daily prebiotic doses of fructooligosaccharides (FOS) and inulin (INU) from Jerusalem artichoke meal (JAM) by improving insulin sensitivity and thus postprandial (ppr.) glucose clearance following a starchy meal. The aim of the recent study was to investigate whether a daily supply of prebiotic doses of FOS+INU *via* JAM alters the ppr. glycemic and insulinemic response of adult healthy nonobese warm-blooded mares with no known history of laminitis.

Methods: During 2 x 3 weeks, 6 healthy warm-blooded mares with no known history of laminitis (age 6 - 13 years; body weight [BW] 519 ± 38.7 kg; body condition score 5.1 ± 0.49/9) were fed crushed oat grains and meadow hay (1 and 15 g/kg BW d^{-1}) in 2 equal meals per day to meet the energy requirement (2). They further received either 0.15 g FOS+INU/kg BW d^{-1} *via* JAM or maize cob meal without grains as placebo (CON). At the end of each period, blood was taken from the *V. jugularis* -60, 0, 30, 60, 90, 120, 180, 240 and 300 min relative to the concentrate meal. 60 min prior that meal 1 kg of hay was provided. Plasma glucose (Hitachi 912), and serum insulin (Insulin-CoA-ACount-RIA-Kit) were measured and areas under the curve (AUC) calculated until 120 and 300 min ppr. (AUC_{120} and AUC_{300}). Statistical analysis was performed using PROC MIXED (two-way ANOVA with fixed effects, SAS, version 9.4) with $P < 0.05$ as level of significance.

Results: The fasting and basal (- 60 min and 0 min prior to the concentrate meal) concentrations of plasma glucose and serum insulin were not influenced by the dietary treatment. Feeding of JAM *vs.* CON did also neither change the ppr. peak of plasma glucose or serum insulin nor the respective AUC´s until 120 and 300 min ppr. (tab.; $P > 0.05$).

LSmeans ± SE	CON	JAM	P value
glucose			
peak [mmol/L]	7.0 ± 0.87	6.3 ± 0.40	0.560
AUC_{120} [mmol/L * min^{-1}]	1,015 ± 41.6	997 ± 41.6	0.783
AUC_{300} [mmol/L * min^{-1}]	2,115 ± 142.3	1,943 ± 142.3	0.464
insulin			
peak [nmol/L]	0.476 ± 0.082	0.508 ± 0.087	0.836
AUC_{120} [nmol/L * min^{-1}]	42 ± 6.3	49 ± 6.3	0.545
AUC_{300} [nmol/L * min^{-1}]	106 ± 14.8	94 ± 14.8	0.673

Conclusion: The results of the study indicate that prebiotic doses of FOS+INU from JAM do not alter the ppr. glycemic and insulinemic response of adult healthy, nonobese warm-blooded mares with no known history of laminitis.

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92.

Influence of vitamin D and 25-hydroxycholecalciferol (25-OHD_3) on RNA expression of P-glycoprotein (P-gp) in the rat

Einfluss von Vitamin D und 25-OHD_3 auf die RNA Expression von P-gp bei Ratten

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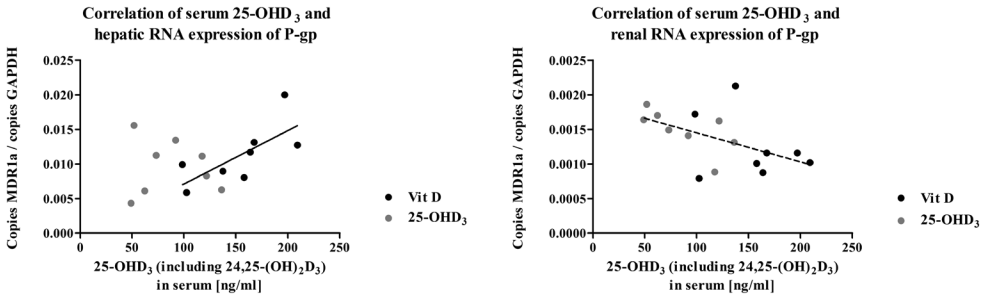
Vitamin D and its metabolite 25-OHD_3 are widely used for prevention of hypocalcemia and as feed additives in livestock. In humans, it has been shown that changes in vitamin D status are associated with fluctuations of plasma concentrations of therapeutic agents that are substrates for P-gp (1), an important apical efflux pump for xenobiotics with a broad substrate spectrum. Furthermore, treatment with 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) resulted in an increase of renal RNA expression of P-gp in rats (2). However, administration of 1,25-$(OH)_2D_3$ and 25-OHD_3 did not affect renal P-gp expression in sheep, while hepatic P-gp protein expression and jejunal P-gp RNA expression was even decreased with 25-OHD_3 supplementation (3). The aim of the current study was to investigate the effect of vitamin D and 25-OHD_3 on renal, hepatic and intestinal P-gp expression in rats.

Animals and Methods: Four groups of female Sprague Dawley® rats (n = 8) received either 25-OHD_3 (6 µg/kg body weight) daily by gavage for ten days, vitamin D (300 µg/kg body weight), once ten days before sacrifice i.m., or a respective placebo. Serum was analysed for 25-OHD_3 using a commercial ELISA (Immundiagnostik AG). RNA was isolated from renal, hepatic and intestinal tissues and expression of P-gp was determined by quantitative RT-PCR. Means were compared by Student´s t-test. Linear regression analysis was carried out to reveal correlations between plasma concentrations of 25-OHD_3 and other parameters. Level of significance was set to $P < 0.05$.

Results: Serum concentration of 25-OHD_3 was significantly increased by either treatment. Although no differences in mean RNA expression of P-gp could be observed between the two groups, a positive linear correlation between serum 25-OHD_3 and hepatic P-gp RNA expression was found in the group treated with vitamin D ($P < 0.05$; $r^2 = 0.52$). Interestingly, a negative correlation was revealed for renal P-gp expression of animals with elevated 25-OHD_3 plasma concentrations due to either treatment ($P < 0.05$; $r^2 = 0.28$).



Conclusion: Treatment with vitamin D or 25-OHD_3 seems to influence P-gp expression in a tissue- and metabolite-specific manner in the rat. As altered expression and thus activity of P-gp can have an impact on elimination of therapeutic agents and xenobiotics, further studies are needed to clarify whether supplementation with vitamin D or its metabolites can lead to side effects.

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93.

Effect of dietary organic acid salts Potassium diformate (KDF) and sodium diformate (NDF) on growth performance and feed utilization of Red Tilapia (*O. niloticus* X *O. mossambicus*) fish grown in brackishwater

Wirkung der organischen Säuresalze Kaliumdiformiat (KDF) und Natriumdiformiat (NDF) im Futter auf Wachstumsleistung und Futterverwertung von Red Tilapia (O. niloticus X O. mossambicus) bei Haltung im Brackwasser

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New strategies are required for feed additives in fish feeds, since the EU-ban of feed antibiotics beginning in 2006. Additives of organic acids are mostly used for feed conservation, but have also demonstrated additional growth-promoting effects in animal nutrition. Red tilapia is a promising fish nowadays in Egypt, especially after the expected shortage in freshwater available for tilapia farming. The addition of organic acids in fish feeds proofed its contribution to promoting fish growth in freshwater ecosystem Liebert et al., (2010). The present study was conducted to examine the efficiency Potassium diformate or Sodium diformate when added separately to low fishmeal Red Tilapia fish diets grown in brackishwater.

Methods: A growth trial (56d) was conducted with Red Tilapia (initial BW 10.2g) making use of the following pelleted basal diet:

Table 1. Formulation of the basal diets used in the present study.

5% fishmeal	10% rice bran	0.5 %Cr_2O_3
35% soybean meal	3% oil (soybean oil:fish oil=1:1)	Ad 100 starch
20% corn	2% premix (vitamins, trace elements)	
10% wheat gluten	1% Dicalcium Phosphate	
10% wheat bran	2% CMC	

Diets contained in DM approx. 33% CP, 7% EE, 6% CF and 440 GE/kcal/100g.

Table 2. The dietary treatments under study

Diet 1	Diet 2	Diet 3
Control	+0.3% K-diformate	+0.3% Na-diformate

Red Tilapia Fingerling was obtained from private hatchery Elkantara, Ismailia, Egypt. Totally four tanks of a semi-closed recirculating system per diet were utilized (25 fish/tank; 250l/tank). The experiment started following an adaptation period of 2 weeks. Fish were fed by hand feeding up to apparent satiation three times a day. Feed intake was recorded biweekly. Apparent protein digestibility was measured according to Furukawa and Tsukahara, (1966). Pooled samples for each diet (3 fish/tank) were analyzed for body composition. Data analysis utilized an exponential N-utilization model according to LIEBERT and BENKENDORFF (2007). Data were submitted to ANOVA ($p<0.05$).

Results: Growth performance and feed utilization of Red Tilapia were significantly improved ($P<0.05$) between treatments receiving organic acid salts compared to control diet as shown in (Table 3).

Table 3.Summary of the results obtained by the presents study.

Parameter	Diet 1 Control	Diet 2	Diet 3
Feed intake (g/fish)	60.45^{b} ±2.4	66.25^{a} ±2.1	66.85^{a} ±1.9
BW gain (%)	403^{b}±8.8	488.4^{a} ±6.7	467.9^{a} ±6.6
SGR (%/d)	2.88^{b} ±0.06	3.16^{a} ±0.04	3.10^{a} ±0.04
Feed conversion ratio(g/g)	1.50^{b} ±0.85	1.35^{a} ±0.29	1.39^{a} ±0.69
Protein efficiency ratio (g/g)	2.22^{b} ±0.85	2.44^{a} ±0.29	2.40^{a} ±0.69
Protein reteantion efficiency (%)*	31.0^{b} ±0.85	34.5^{a} ±0.29	34.8^{a}±0.69
Appearent protein digestabiliy (%)	78.9^{b} ±0.85	84.7^{a} ±0.29	84.1^{a} ±0.69
Protein quality (model parameter, relative)	100	112	112

*CP-deposition : CP-intake (%)

Conclusion: The addition of low levels of the organic acid salts KDF and NDF have significant effects on the overall culture performance of fingerling Red tilapia, with better final weights, growth rates and feed utilization. Present results therefore call for the inclusion of organic acid salts, like potassium- or sodium-diformate, in the formulated feeds for farmed Red tilapia under commercial field conditions.

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94.

Effects of feeding high-quality hay with graded amounts of concentrate on the bacterial community structure of solid and liquid rumen content in cows

Einfluss der Fütterung von qualitativ hochwertigem Heu mit unterschiedlichen Mengen an Kraftfutter auf die Bakterien im flüssigen und festen Panseninhalt von Kühen

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Feeding of high-grain diets to dairy cattle has become a common strategy to increase milk production, but negative side-effects on rumen and metabolic health can result due to quick fermentation of starch. With their rumen microbial ecosystem dairy cows are efficiently adapted to fibre-based diets; therefore the aim of this study was to replace grain-based concentrate by high-quality hay (in dry matter (DM) 18.7% sugar and 23.5% crude protein (CP)) and to evaluate the effects of varying high-quality hay to concentrate ratios on the bacteria both in the free ruminal liquid and attached to particles in the solid rumen digesta.

Methods: In a double 4 x 4 Latin Square 8 rumen cannulated non-lactating cows were fed 60HQ (60% high-quality hay and 40% concentrate), 75HQ (75% high-quality hay and 25% concentrate), and 100HQ (100% high-quality hay). A fourth diet with 60% fibre-rich hay and 40% concentrate (60NQ) was considered as control. Fibre-rich and high-quality hay differed in contents of sugar (in DM 11.3% vs. 18.7%) and neutral detergent fibre (NDF in DM 58% vs. 46%). Accordingly sugar content of the diets ranged from 8.4% to 18.7% and starch from 0% to 18.2% in DM. Each run lasted 25 days, with days 1 to 6 used to stepwise switching the diet, and days 7 to 25 considered as experimental period. Free ruminal fluid (FRL) from the ventral rumen and solid digesta from the fibre mat were taken 2h after the morning feeding on day 24 and DNA was extracted using the MOBIO PowerSoil® DNA Isolation Kit with adaptations for the rumen ecosystem. Amplicon sequencing targeting the V345 region of the 16S rRNA gene was done using Illumina MiSeq paired-ends sequencing technology (Microsynth AG) and data were analyzed with QIIME. Quality control was used to remove chimeras and false-positive identification of Operational Taxonomic Units (OTUs) clustered to a 97% identification with a minimum of 10 sequences per OTU. Statistical analyses were performed using Proc princomp and Proc Corr of SAS.

Results: Principle component analysis revealed different clustering of OTUs between FRL and digesta. Phyla in FRL were identified as mainly *Firmicutes, Proteobacteria* and *Bacteroidetes*. Whereas main phyla of particle attached bacteria were identified as *Bacteroidetes*, *Fibrobacteres* and *Firmicutes*. In FRL abundance of *Proteobacteria* negatively correlated with sugar intake ($r = -0.572$, $P<0.001$) and positively correlated with starch intake ($r = 0.458$, $P<0.05$). In solid digesta samples numbers of OTUs belonging to *Synergistetes* ($r = -0.487$, $P<0.05$), *Cyanobacteria* ($r = -0.605$, $P<0.001$) and *Fibrobacteres* ($r = -0.406$, $P<0.05$) negatively correlated with sugar intake. *Fibrobacteres* phylum was unaffected by starch intake, whereas numbers of OTUs belonging to the phyla *Synergistetes* ($r = 0.484$, $P<0.05$) and *Cyanobacteria* ($r = 0.482$, $P<0.05$) increased with increasing starch intake. In solid digesta samples on the taxa family level *Ruminococcaceae* positively correlated with sugar ($r = 0.836$, $P<0.001$) and negatively correlated with starch intake ($r = -0.771$, $P<0.001$). *Prevotellaceae* also positively correlated with sugar intake ($r = 0.515$, $P<0.05$), whereas numbers of *Dethiosulfovibrionaceae* decreased with increasing sugar intake ($r = -0.496$, $P<0.05$) but positively correlated with starch intake ($r= 0.495$, $P<0.05$). In FRL *Christensenellaceae* decreased with increasing starch intake ($r = -0.387$, $P<0.05$) whereas *Succinivibrionaceae* increased ($r = 0.468$, $P<0.05$) but negatively correlated with sugar intake ($r = -0.566$, $P<0.05$). Thus, a higher abundance in FRL of *Succinivibrionaceae* was found with 60NQ than with 60HQ.

Conclusion: The free ruminal liquid was dominated by the *Firmicutes* phylum, whereas in the solid digesta the majority of bacteria belonged to the phylum *Bacteriodetes*. Ruminal microbes were differently affected by starch and sugar intake, whereby effects were more pronounced in microbial populations attached to particles than in the free ruminal liquid.

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95.

Effects of feeding high-quality hay with graded amounts of concentrate on dry matter intake, milk yield and milk composition of early lactating dairy cows

Einfluss der Fütterung von qualitativ hochwertigem Heu mit unterschiedlichen Kraftfutterniveaus auf die Trockenmasseaufnahme, Milchleistung und Milchzusammensetzung früh-laktierender Milchkühe

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Introduction: Feeding of starch-rich concentrates at the expense of fiber-rich forages increases the risk for ruminal disorders. A viable alternative to concentrate feeding in dairy cattle might be the feeding of high-quality hay, i.e. a roughage rich in energy in form of water soluble carbohydrates and crude protein (CP) that could ideally maintain milk production and support ruminal health. Thus, the aim of this study was to evaluate the effects of feeding high-quality hay alone or with graded amounts of concentrate on dry matter intake (DMI), milk yield, milk composition and fat corrected milk (FCM) in a feeding experiment with 24 Simmental cows in early lactation.

Methods: Four different diets (dry matter (DM) basis), 60% normal-quality hay and 40% concentrate (60NQ), 60% high-quality hay and 40% concentrate (60HQ), 75% high-quality hay and 25% concentrate (75HQ), and 100% high-quality hay (100HQ), were fed to 24 Simmental cows (per group n=6), starting from 7 days ante partum until 28 days post partum. Cows were blocked by lactation number and milk yield of the last lactation. High-quality hay and normal-quality hay contained on average ~19% and ~11% sugar as well as ~18% and ~7% CP, respectively. Feed intake and milk yield were recorded daily. Milk samples were collected on day (d) 7, d 14, d 21, and d 28 to analyse milk composition. The FCM was calculated (on 4% Fat-basis) as kg FCM = milk yield (kg) · [(fat% · 0.15) + 0.4] (1). Statistical analysis was performed using the MIXED procedure of SAS.

Results: DMI and milk yield increased continuously over the 28-d feeding period, and in week 4 post partum DMI was highest in Groups 60HQ and 75HQ and milk yield highest in Group 60HQ ($P \leq 0.05$). Group 75HQ displayed higher milk protein contents than the other groups ($P \leq 0.05$). Milk urea was highest in Group 100HQ compared to the other groups ($P \leq 0.05$). No differences were shown in the FCM, milk fat and milk lactose between the experimental groups.

Table 1 Dry matter intake (DMI), milk yield and milk composition of the experimental groups

	Experimental groups			
Week 4	60NQ	60HQ	75HQ	100HQ
DMI [kg/day]	$21.4^{ab} \pm 1.20$	$22.3^{a} \pm 1.30$	$22.6^{a} \pm 1.20$	$18.6^{b} \pm 1.18$
Milk yield [kg/d]	$35.1^{b} \pm 2.19$	$40.4^{a} \pm 2.18$	$34.0^{b} \pm 2.19$	$34.0^{b} \pm 2.24$
FCM [kg/d][1]	35.1 ± 4.26	43.6 ± 4.23	33.4 ± 4.25	43.3 ± 5.03
Fat [%][1]	4.01 ± 0.63	4.66 ± 0.63	3.93 ± 0.63	5.70 ± 0.77
Protein [%][1]	$2.86^{b} \pm 0.09$	$3.04^{b} \pm 0.09$	$3.33^{a} \pm 0.09$	$2.93^{b} \pm 0.11$
Lactose [%][1]	4.78 ± 0.06	4.71 ± 0.06	4.74 ± 0.06	4.60 ± 0.07
Urea [mg/100 ml][1]	$30.8^{ab} \pm 4.84$	$17.0^{c} \pm 4.82$	$26.9^{b} \pm 4.84$	$44.4^{a} \pm 5.20$

LS-Means (± SEM); [1] FCM and milk composition relate to sampling on d 28, values with different superscripts within a row differ statistically ($P \leq 0.05$).

Conclusion: Results show that milk yield can be significantly improved when normal fiber-rich hay is replaced by high-quality hay. FCM yield in Group 100HQ was higher and comparable to FCM yield in Group 60HQ. As crude protein content in the high-quality hay was high, higher urea content in milk was expected in Group 100HQ. Further analyses of blood parameters have to show to which extent the negative energy balance does account for the lower protein and higher urea content in milk, and if high-quality hay alone can be a healthy feeding option during the transition period.

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96.

Effects of sugar beet silage on ruminal fermentation *in vitro*

Effekte von Zuckerrübensilage auf ruminale Fermentationscharakteristika in vitro

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Question: Conserving sugar beets (*Beta vulgaris*) as silage represents a possibility to preserve beets. But as this silage still has a relatively high content of sucrose, its feeding may increase the risk of subacute ruminal acidosis. So the aim of the present study was to test if the inclusion of sugar beet silage (SBS) in a total mixed ration (TMR) altered pH and volatile fatty acid (VFA) concentrations in a Rusitec system. Branched-chain VFA where chosen as indicative for N catabolism.

Methods: During initial adaptation the Rusitec fermenters were supplied with 10.6 g dry matter (DM) of grass hay and 4.5 g DM of a commercial concentrate (CON). The basal experimental diet was added from day 8 on and consisted of maize silage, grass silage, straw and soybean meal. Either 0 (SBS-0), 12.5 (SBS-12.5) or 25 (SBS-25) % of this ration (DM basis) were replaced with SBS (DM 213 g/kg; crude protein 56, sugar 203 g/kg DM). Each ration was run in duplicate in 2 separate 17 days-lasting runs and allocation to fermenters was completely randomized. The incubation procedure was done as described by (1), buffer according to (2) was infused at a rate of 0.029/hour. On days 6, 10 and 15 pH was measured immediately before feeding and 2, 4, 8, 12 and 24 hours after feeding. Samples for VFA analysis were taken at the same time. Data were grouped by day and dietary treatment and subjected to a univariate analysis of variance followed by the LSD or the Games-Howell test as post hoc tests. Comparison between days was done using the t-test for dependent samples.

Results: There were no differences in pH and VFA levels on day 6 between the respective fermenters (individual data not shown). On day 10 SBS-25 decreased pH but total VFA were not affected (Table 1). Only i-valerate was increased by SBS. Five days later pH was still lower with SBS-25 and propionate was now lower with both SBS-containing TMR. Mean i-valeriate concentration had increased for all dietary treatments and was still higher with SBS.

Table 1: Effects of sugar beet silage on pH and VFA (mmol/L) in a Rusitec system (means)

Variable	Experimental day, dietary treatment						
	Day 6	Day 10			Day 15		
	CON	SBS-0	SBS-12.5	SBS-25	SBS-0	SBS-12.5	SBS-25
pH	6.65	$6.53^{a,x}$	6.53^{a}	6.49^{b}	$6.58^{a,y}$	6.56^{a}	6.50^{b}
Acetate	55.7	52.7	51.6	56.9^{x}	50.8	50.6	51.6^{y}
Propionate	22.8	23.1^{a}	$19.4^{b,x}$	$20.8^{a,b,x}$	21.8^{a}	$16.3^{b,y}$	$15.4^{b,y}$
Butyrate	15.5	$24.9^{a,x}$	21.7^{b}	$23.2^{a,b}$	20.5^{y}	20.5	21.1
Valerate	2.08	$2.79^{a,x}$	$2.85^{a,b,x}$	$3.13^{b,x}$	2.21^{y}	2.16^{y}	2.14^{y}
i-Butyrate	0.79	1.04	0.83	0.92	0.92^{a}	0.78^{b}	0.72^{b}
i-Valerate	1.96	$4.16^{a,x}$	$5.08^{b,x}$	$5.33^{b,x}$	$4.73^{a,y}$	$8.04^{b,y}$	$9.12^{b,y}$
∑ VFA	94.0	108^{x}	101	110^{x}	101^{y}	98.4	100^{y}

^{a,b} Means within a day differ; ^{x,y} treatment means differ between days 10 and 15; $p < 0.05$

Conclusion: A percentage of 25% SBS in the ration admittedly did not affect total VFA concentration but decreased pH even in the well- and continuously-buffered Rusitec system. Distinctly increased concentrations of i-valerate derived from amino acid catabolism with SBS-containing TMR indicate that the effect may be linked to ruminal protein or amino acid catabolism, respectively, and is probably based on less protein-based buffering capacity.

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97.

Feed sorting, eating behavior and production performance of early-lactating cows differing in responsiveness of ruminal pH to high concentrate feeding

Futterselektions- und Fressverhalten, sowie Produktionsparameter von frisch laktierenden Kühen mit unterschiedlicher ruminaler pH-Sensitivität gegenüber hohen Kraftfuttergaben

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It is well recognized that cows can differ greatly in their susceptibility to subacute ruminal acidosis (SARA) even when fed and managed similarly. An adaptation of the rumen to fermentation acids in the rumen can only partly explain such divergence (1) and other underpinning causes have to be found. This study focused on eating and chewing behavior and production performance of early lactating cows with different ruminal pH as affected by an intermittent feeding of a high concentrate diet. We expected that cows will not always select the diet to reduce effects of low ruminal pH and specific eating behaviors might determine whether or not they are susceptible to SARA.

Methods: Eighteen early-lactating Simmental cows (66.6±20.45 days in milk, mean±SD) balanced for the lactation number and days-in-milk were assigned to 2 feeding groups: control (n=6) and high-concentrate (HC; n= 12). All cows started at baseline (Base) and were fed for 7 days a total mixed ration (TMR) containing 27% concentrate (dry matter basis) plus an additional concentrate to reach a concentrate level of 40%. The control cows continued with this feeding regime for 29 days. The HC cows were challenged with a 60%-concentrate TMR for 8 days to induce SARA followed by a 7-day break when they were fed similarly to the control cows. Thereafter they were re-challenged for another 14 days. Ruminal pH (wireless sensors), intake and milk yield were continuously monitored throughout the experiment. Milk components were analyzed weekly. Chewing activity was monitored using a noseband sensor (RumiWatch system, ITIN + Hoch GmbH). Feed sorting index was estimated from relative changes in the particle size distribution of the offered and unconsumed TMR collected from individual cows. The top 6 cows in the HC group with the most severe pH suppression were grouped as susceptible and the remaining six cows as tolerant cows prior to statistical analysis. Data of the control, susceptible and tolerant cow groups during Base and SARA periods were analyzed using the MIXED procedure of SAS.

Results: The lactation number, day in milk and body weight at start (average ± SD) of the control cows were 2.3 ± 1.8, 68 ± 12.9 days and 657 ± 91 kg, respectively, whereas those of susceptible group were 2.8 ± 2.1, 48 ± 22.6 days and 748 ± 110 kg, respectively and 2.5 ± 1.9, 78 ± 13.0 days and 732 ± 94 kg, respectively for the tolerant group. Independent of dry matter intake, only cows in the susceptible group experienced SARA. The greater severity of ruminal pH suppression was already evident in Base and became more intense during SARA challenge, especially in the first week. Daily milk yield and the concentration of fat, protein and lactose during Base and SARA periods were similar among all cow groups. No major difference in the sorting index was observed. But the susceptible group regurgitated about 2-3 boli per hour less than the control and tolerant groups in both Base and SARA periods (P<0.05). The susceptible group chewed more per swallowed bolus (54 in Base and 66 in SARA) compared to the other two groups (50 in both periods) (P<0.05). During SARA period the susceptible group had shorter eating time (min/h) and lower eating chews (n/h) than the other groups at about 2-3 h after the morning feeding (P<0.05). Overall, there was no change in rumination time with respect to cow group and feeding period.

Conclusions: Based on the feeding management and SARA conditions, the cows did not sort the TMR. Regardless of the diet, the susceptible cows had a lower number of feed boli swallowed and they appeared to chew more per swallowed bolus, however this was not sufficient to counterbalance the low ruminal pH they experienced.

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A meta-analysis of the effect of sugar beet pulp as a feed for dairy cows on dry matter intake, production performance and net food production

Meta-Analyse des Effekts von Trockenschnitzeln als Futter für Milchkühe auf Futteraufnahme, Milchleistungsparameter und Netto Nahrungsmittelproduktion

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Introduction: Replacing human edible concentrates with agro-industrial by-products such as sugar beet pulp (Bp) is an economically viable option in dairy cattle production to positively affect the net food production, i.e. the produced food in relation to the potentially human edible feed input (1). However, high inclusion rates might impair feed intake and performance of the cows. The present meta-analysis (2), based on studies published in the past 3 decades, was performed to evaluate inclusion of Bp in dairy cow diets taking into account inclusion rate of Bp, feed intake and production levels of the cows, concentrate level in the diet as well as the replacement aim of Bp in the diet (i.e., grain type and forage replaced in the diet).

Methods: A database with a total of 104 treatments from 34 studies was constructed from literature published from 1990 to present. Dependent variables included in the database were dry matter intake (DMI), performance data and the human edible energy (heE) input (1). Treatments were categorized based on the Bp inclusion levels in to 4 groups: "Zero" (0% Bp), "Low" (0.1 - 10% Bp), "Medium" (10.1 - 20% Bp) and "High" (>20% Bp) on a dry matter basis. The mentioned variables were analyzed to evaluate the effects of the Bp inclusion levels. Meta-regression approaches were used to determine the effects of Bp replacement aim (Barley vs. Maize vs. Others) on DMI, milk production variables as well as on net food production (kg FCM/MJ heE input) as a measure of sustainability. For determination of the Bp effect on feed intake level, a regression of these variables was evaluated for two discrete intake levels (low vs. high, with the cut-off margin of 3.5% BW). Meta-analysis and meta-regression were performed using modelling approaching implemented in SAS.

Results: The use of Bp had no effect on overall DMI (P=0.259), but negatively affected NE_L intake (P=0.009), while increasing NDF intake (P<0.001). Bp feeding did not affect milk yield but increased milk fat yield and FCM (P<0.01) with the Medium Bp group resulted in the highest fat yield and FCM. Milk lactose and protein yield was unaffected by Bp inclusion. The effect of Bp was found to be dependent on the DMI level of the cows and the inclusion of Bp (%) was more likely to suppress DMI (% of BW) when the intake level was >3.5% (regression equation: DMI = 3.63 - 0.009 × Bp and P_{slope}=0.037), while in cows with a DMI ≤3.5% of BW Bp was more likely to increase DMI (regression equation: DMI = 3.21 + 0.003 × Bp and P_{slope}=0.145). The net food production strongly increased from 0.24 kg FCM/MJ heE input in the Bp group "Zero" to 1.38 kg FCM/MJ heE input in the Bp group "High" (P<0.01). The variables studied were not affected by the replacement aim.

Conclusions: The feeding of Bp can improve sustainability in dairy industry by increasing the net food production. Data suggest a lipogenic effect of Bp on milk component synthesis. While the effect of Bp on the DMI is believed to be attributed to the Bp feeding level, our meta-analysis indicates that the effect of Bp on the DMI depends on the cow's feed intake level and therefore on the targeted production level. Accordingly, high inclusion rates of Bp seem to be particularly beneficial in cows in which the feed intake level does not exceed 3.5% of BW. The improved feed efficiency was linked to potentially higher inclusion rates of easily digestible fiber when Bp was fed.

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99.

Investigations on the ruminal fermentation of untreated and cell disrupted microalgae *in vitro*

Untersuchungen zur ruminalen Fermentation von unaufgeschlossenen und aufgeschlossenen Mikroalgen in vitro

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Microalgae are a promising potential feed resource due to their favourable nutrient composition. However, their nutritional value may vary between algae species and also within species depending on cultivation conditions. Furthermore, many microalgae species develop robust cell walls or cell coverings which may restrict nutrient digestibility. Data on the ruminal fermentation of microalgae are scarce. Thus, it was the objective of this study to investigate if there are differences in ruminal fermentation characteristics of different microalgae species and if cell disruption affects ruminal fermentation.

Methods: Commercially available microalgae biomass of the four genera *Arthrospira* (n=2), *Chlorella* (n=8), *Nannochloropsis* and *Phaeodactylum* (n=2) was assayed using the Hohenheim Gas Test [1]. A subset of each sample was treated with a stirred ball mill for cell disruption resulting in a total of 32 samples for *in vitro* incubations. Each sample was tested in six consecutive runs and gas production was recorded after 2, 4, 6, 8, 12, 24, 32, 48 and 72 h of incubation. An exponential model was fitted to the data for estimating the parameters b (potential gas production, ml/200 mg dry matter (DM)) and c (rate constant of gas production, %/h) using Proc NLMIXED of SAS. In addition, a comprehensive characterization of the chemical composition (proximate nutrients, starch, amino acids, minerals, and fatty acids) of the microalgae biomass was performed. Correlation analysis (Proc CORR) was used to study relationships between gas production parameters and chemical constituents of the microalgae.

Results: The samples had an overall low level of potential gas production with a considerable variation between and within genera (Table). Cell disruption affected the gas production parameters b and c, but extent and direction of the effect varied appreciably. Amongst others, a positive correlation was found between b and the concentration of crude protein (r=0.57) and starch (r=0.83) and a negative correlation was found between b and the concentration of crude ash (-0.61) and eicosapentaenoic acid (-0.66).

Table: Potential gas production (B) and rate constant of gas production (C) of untreated (ut) and cell disrupted (cd) microalgae biomass

		Arthrospira		***Chlorella***		***Nannochloropsis***		***Phaeodactylum***	
		(n=2)		(n=8)		(n=4)		(n=2)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
B	ut	26^{aA}	23-30	26^{aB}	14-35	17^{bB}	14-20	13^{cB}	9-18
(ml/200 mg DM)	cd	25^{bB}	22-28	33^{aA}	23-41	19^{cA}	15-23	17^{dA}	14-20
C	ut	14^{cA}	14-15	9^{dB}	5-21	15^{bB}	8-23	36^{aA}	26-46
(%/h)	cd	12^{dB}	11-13	14^{cA}	9-20	28^{aA}	24-30	24^{bB}	21-24

$^{a-d}$ Different superscript letters within a row indicate significant mean differences (p<0.05).

$^{A, B}$ Different superscript letters within a column indicate a significant treatment effect (p<0.05).

Conclusions: The ruminal fermentation of microalgae varies greatly between and within microalgae genera which may result from differences in the cultivation conditions which are not standardized. Thus, a generally applicable nutritional value of microalgae cannot be given. Further studies are necessary to evaluate the effect of the cultivation conditions on the nutritional value of microalgae. Mechanical cell disruption is a possible treatment to enhance ruminal fermentation of *Chlorella, Nannochloropsis and Phaeodactylum* but disruption appears not be necessary for *Arthrospira.*

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100.

Effects of varying supply of fibre, starch and sugar for fattening bulls on feed intake and fattening performance

Einfluss einer unterschiedlichen Versorgung mit Faser, Stärke und Zucker in Rationen für Mastbullen auf die Mast- und Schlachtleistung

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In contrast to dairy cows in which adequate supply of structural fibre and ruminally degradable starch and sugar is presently in focus, there is only scarce information in fattening bulls in this respect. Former studies showed only minor effects when dietary structural value (SV) was reduced from 1.2 to 0.6 (1) so that fibre requirement in fattening bulls appears to be very low compared to requirements of dairy cows. In this study, however, relatively high amounts of sugar beet pulp were fed resulting in comparably low levels of dietary SV, which may have lowered possible effects on ruminal fermentation. For this reason, a feeding trial was conducted to investigate effects of diets varying in structural value and source of carbohydrates on performance of fattening bulls.

Methods: 71 growing German Simmental bulls (Body weight (BW): 238 ±21 kg; age: 162 ±6 days (d)) were assigned equally to one of three dietary treatment (treat) groups. Treat SV 1.0 were fed for ad libitum intake a Total Mixed Ration (TMR) based on maize silage (68 % of dry matter (DM)), and concentrates (32 % of DM). Treat SV 0.52 was fed a TMR composed of 31 % maize silage and 69 % concentrates in DM, containing a portion of 36 % pressed beet pulp. In concentrates of treat SV 0.48, portion of sugar beet pulp in concentrates was reduced to 22 % of DM, but portion of barley was increased. Dietary concentration of peNDFom > 1.18 was 307, 235, and 211 g/kg DM for treat SV 1.00, 0.52 and 0.48, concentration of starch + sugar was 366, 404, and 438 g/kg DM. Individual feed intake was automatically recorded daily while BW was recorded every four weeks. Reticuloruminal pH of 8 animals per treat was measured by an indwelling and wireless data transmitting unit (smaXtec animal care sales GmbH, Austria). The bulls were slaughtered at an average age of 478 days. The data were evaluated by a one-factorial ANOVA using SAS. Data of 23, 22, and 24 animals were used for groups SV 1.0, 0.52, and 0.48.

Results: Daily DM, ME and starch+sugar intake increased when SV of the diet decreased (table). Final weight and daily gain tended to be depressed in treat SV 0.48. Considering threshold levels of ruminal pH for dairy cows there was no indication for increasing risk for SARA with decreasing SV of the diet. There were only minor differences in carcass quality traits between treatments, but back fat depth at slaughter was increased ($p<0.05$) and intramus under fat content (IMF) tended ($p<0.1$) to be increased in treat SV 1.0.

Treatment*:	SV 1.0	SV 0.52	SV 0.48
Feed intake, kg Dry Matter/day	9.5±0.8^{b}	10.1±0.7^{a}	10.2±0.9^{a}
Metabolizable Energy intake, MJ /day	110±9^{b}	122±8^{a}	125±11^{a}
Starch+Sugar intake, g/ayd	3450±291^{c}	4072±275^{b}	4434±404^{a}
Amylase treated Neutral detergent fibre, ash-free (aNDFom) intake, g/d	3396±285^{a}	3179±214^{b}	3020±276^{c}
Final body weight, kg	796±77	793±50	771±45
Daily gain, g	1771±193	1765±115	1695±141
Back fat depth at slaughter, cm	2.7±0.6^{a}	2.2±0.5^{b}	2.3±0.4^{b}
Intramuscular Fat content, %	4.1±1.2	3.5±1.2	3.4±1.2
Metabolizable Energy/gain, MJ/kg	62±5^{c}	69±5^{b}	74±6^{a}

*Means ± Standard Deviation; $^{a, b, c}$indicate significant differences ($p<0.05$) between treat means.

Conclusions: Despite the high level of performance in all feeding groups, decreasing dietary SV clearly depressed ME- and DM- utilization for growth presumably due to an impaired ruminal fermentation. Level of feed intake was comparable in feeding groups SV 0.52 and 0.48, but daily gain tended to be lower in feeding group SV 0.48. In conclusion, a dietary SV of about 0.5 seems to represent a threshold value to fattening bulls fed rations with high portions of rapidly and highly degradable starch and of sugar.

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101.

Impact of low- and medium-oil corn dried distillers' grains with solubles on growth performance of feedlot cattle

Einfluss getrockneter Maisschlempen mit mittlerem und niedrigem Fettgehalt auf die Mastleistung von Fleischrindern

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Introduction: Feeding conventional corn-based dried distillers' grains with solubles (DDGS) to ruminants frequently has a positive impact on growth performance. Since corn DDGS has a relatively high oil content and corn oil can be used for biodiesel production, ethanol producers use enhanced oil extraction technologies to recover oil from DDGS. Corn distillers' oil (CDO) is typically removed mechanically while solvent-based extraction is used less frequently. Differences in CDO extraction technology have led to variation in DDGS composition. This study was designed to examine the effect of low-oil DDGS (LO-DDGS, 5.6% oil) or medium-oil DDGS [MO-DDGS, 8.3% oil; all dry matter (DM) basis] on feed intake, growth performance and carcass quality of beef steers fed corn silage-barley grain diets.

Methods: A combined growing (84 days (d)) and finishing (112 d) study was conducted using 160 Angus crossbreed steers. Steers were blocked by weight and randomly assigned to 1 of 16 pens (n = 10 per pen). Each pen was randomly allocated to 1 of 4 diets containing 10% or 20% of LO-DDGS (5.6% oil), or MO-DDGS (8.3% oil) during growing phase (all DM basis). Treatment allocation was maintained in the finishing phase while the inclusion level of DDGS was reduced from 10% to 5% and 20% to 10% DM to adjust for the lower protein requirements of finishing cattle. Distillers' grains in all diets partially replaced barley grain. Steers were fed once daily targeting

Results: Feeding LO-DDGS diets during the growing period increased DM intake (DMI, P 0.10). However, feeding MO-DDGS to finishing steers resulted in improved Gain:Feed compared to LO-DDGS (P=0.03).

Table 1. Growth performance of steers fed low- or medium-oil DDGS (LO-DDGS or MO-DDGS).

	LO-DDGS		MO-DDGS			P-value		
Item	10%	20%	10%	20%	SEM	DDGS	Level	DDGS × Level
Growing								
DMI (kg/d)	7.7	8.0	7.5	7.5	0.09		0.13	0.26
ADG (kg)	1.43	1.52	1.39	1.42	0.029	0.03	0.06	0.35
Gain:Feed	0.19	0.19	0.19	0.19	0.003	0.86	0.42	0.55
Finishing	5%	10%	5%	10%	SEM	DDGS	Level	DDGS × Level
DMI (kg/d)	11.5	11.7	11.0	11.4	0.23	0.11	0.24	0.67
ADG (kg)	1.87	1.90	1.88	1.92	0.043	0.71	0.50	0.91
Gain:Feed	0.16	0.16	0.17	0.17	0.003	0.03	0.99	0.87

Conclusion: In growing cattle LO-DDGS stimulated DMI which led to improved ADG compared to MO-DDGS. The reason for the observed stimulation of DMI is uncertain. The higher energy density of MO-DDGS only improved feed conversion in finishing cattle. Results indicate that DDGS with higher residual fat content is most efficiently utilized in finishing cattle.

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102.

A targeted metabolomics approach to compare metabolic profiles of calves reared on whole milk or on milk replacer

Vergleich der Stoffwechselprofile in Kälbern die mit Vollmilch oder Milchaustauscher aufgezogen wurden - eine gerichtete Metabolomstudie

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Question: Adequate nutrition of calves is considered to be a fundamental factor for an optimal production in adult age. Previous studies showed that calves fed whole milk ad libitum produced more milk in their first lactation than calves fed milk replacer ad libitum [1], discussed as an imprinting of metabolic function in the calf potentially due to non-nutritive components [2]. However, differential ways of metabolic modulation due to rearing on whole milk vs. milk replacer have not been elucidated yet. Therefore, as a systemic screening, this study performed a targeted metabolomics analysis to compare blood metabolic profiles of calves reared on whole milk vs. milk replacer, in order to generate novel hypotheses regarding affected metabolic pathways.

Methods: German Holstein calves were allocated to two feeding groups: whole milk (WM; n=10) and milk replacer (MR; n=9). All calves received colostrum and transition milk ad libitum for 3 d post natum. From d 4 to d 25 calves were fed according to their grouping either with WM or with MR (13.8 % solids, MR: 23 % CP and 17 % CF) ad libitum (average daily intakes 9.47 and 9.25 kg, respectively). One blood plasma sample was collected from every calf on d 25 post natum for metabolomics analysis. Blood serum samples were subjected to a liquid chromatography - mass spectrometry (LC-MS) based targeted metabolomics analysis using the AbsoluteIDQ p180 Kit of Biocrates Life Science AG (Innsbruck, Austria). Processed metabolomics data were evaluated by heatmap visualization and multivariate data analysis techniques such as principal component analysis (PCA) and partial least squares - discriminant analysis (PLS-DA).

Results: During 4 d and 25 d post natum average daily energy intake (ME) was 27.2±3.3 MJ/d and 20.6±3.8 MJ/d (mean±SD, Px:*y* indicates chain length of *x* and number of unsaturated bonds of *y*), based on the PLS-DA and scores of variable importance in projection (VIP).

Conclusions: Our findings show that calves reared on whole milk and calves reared on milk replacer had dissimilar metabolic profiles. Dissimilarities were due to particular lipid species, the most of which can be associated with membrane function, cellular turnover and signaling cascades. Particularly, these metabolites are known to be important structural components of plasma lipoproteins and cell membranes and are involved in regulation of cell function, membrane protein trafficking and inflammation. However it has to be noted that the main application of this metabolomics approach is to generate new hypotheses, accordingly the underlying physiological mechanisms of the indicated lipid pathways have to be elucidated in future studies.

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103.

Effect of *Birdsfoot trefoil* and dietary protein level on growth performance and fatty acid composition of the intramuscular fat of lamb loins

Einfluss von Hornklee und unterschiedlichem Rohproteingehalt der Ration auf das Wachstum und die Fettsäurenzusammensetzung des intramuskulären Fettes des longissimus dorsi Muskels beim Lamm

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The fatty acid composition of ruminant products is often criticised due to the high content of saturated fatty acids (SFA) as it is related to elevated risks of cardiovascular diseases (1). Therefore, finding ways to increase the content of beneficial polyunsaturated fatty acid (PUFA), especially n-3 and conjugated linoleic acids (CLA) in ruminant source food is an ongoing challenge. Bioactive compounds like condensed tannins (CT) have been reported to protect dietary PUFA from ruminal biohydrogenation, thereby enhancing their availability for absorption in the small intestine resulting in greater incorporation into the adipose tissue (2). Furthermore, it has been shown that dietary CT protect dietary proteins from microbial degradation and thus increase their flow to the duodenum. However, less is known if the extent lower microbial degradation depends on the dietary protein level. Thus, the objective of the present study was to determine the impact of Birdsfoot trefoil (*Lotus corniculatus*), a CT-containing legume included in a high and low protein basal diet on the growth performance, carcass yield and fatty acid composition of the intra-muscular fat of lamb loins.

Methods: Twenty-four White Alpine ram lambs (BW 21.7 ± 2.7 kg) were fed a basal diet consisting of 56% (as DM) Birdsfoot trefoil silage (19.9 g/kg DM of CT) and 44% (as DM) hay. Additionally, the lambs were offered either a barley concentrate to reach the predicted protein requirements (BP) or a concentrate with a crude protein level 20% above the requirements (HP). Polyethylene glycol (PEG) was used in order to neutralize the effect of CT in 2 of the 4 diets: BP+PEG ($BP^{\pm}$) and HP+PEG ($HP^{\pm}$). The experimental diets were offered daily as two equal meals at 07:00 and 15:00. Feed intake and body weight were measured daily and weekly, respectively. Lambs were slaughtered when they reached approximately 40 kg body weight. Carcasses were weighed and then refrigerated at 4 °C for 24 h. The complete *longissimus dorsi* was removed from the left side of the carcasses and stored at 4°C until the fatty acid analyses were performed. The data were analysed with the MIXED procedure of SAS using protein levels (BP and HP), PEG addition (BP^{+} and HP^{+}) and the 1-way interaction as fixed effects.

Results: Lambs fed the HP and HP^{+} diet ingested more feed (28.6%; P+. Consequently, slaughter weight (P+ and BP^{+} lambs. Oleic acid levels in the intramuscular fat of loins from the HP and HP^{+} group were greater (20.7%; PMUFA) compared with the BP and BP^{+} group. Docosapentaenoic and arachidonic acid as well as eicosapentaenoic and α-linolenic acid levels were up to 16.7% lower (P< 0.05) in the intramuscular fat of loins from lambs fed the HP and HP^{+} than the BP and BP^{+} diet. Condensed tannin supplementation reduced (P

Conclusion: The results of this study demonstrated that except for a slight but significant increase of arachidonic levels in the CT groups, supplementation of CT in the diet of lambs was not sufficient to increase the PUFA concentrations in lamb meat. Further research needs to determine at what inclusion level and to what extent different CT sources affect biohydrogenation of dietary PUFA and ultimately their incorporation into the tissues.

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104.

Effects of an additionally ad libitum offered „fibre mix“ in sows on farrowing, colostrum supply and body condition of the sow

Untersuchungen zur Rohfaserergänzung im geburtsnahen Zeitraum - Effekte auf den Geburtsverlauf, die Kolostrumversorgung von Ferkeln und die Konditionsentwicklung von Sauen

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The number of born and weaned piglets per sow has been increased significant over the last years. The hypothesis of this study was that a separate offer of a high fiber diet around parturition followed by an ad libitum feeding concept in lactation leads to beneficial health effects on sows and piglets.

Methods: From day 109 (d-7) of gestation, a total of 25 (n=13 CONTROL and n=12 ADLIB) sows were fed daily two portions of a commercial lactation diet (per kg DM: 190g XP, 48.3g XF, 14.7 MJ ME) until d35 of lactation following a manually controlled restricted feeding scheme with daily 2.4 kg DM a.p. and increasing amounts of DM p.p. until ad libitum feeding from d10 p.p. onwards. The ADLIB was offered an additional feed out of a feeding dispenser containing a fibre-lactation-diet mixture (~85 % fibre pellet - per kg DM: 125g XP, 179g XF, 10.0 MJ ME - and ~15% lactation diet) from d-7 until d2 and from d3 onwards lactation diet. Feed intake of sows was measured daily. Salivary cortisol concentrations around parturition (d-2, 24h, 72h p.p.) were determined using a commercial ELISA Kit (Cortisol free in Saliva ELISA; Demeditec Diagnostics GmbH, Kiel, Germany). Within 24h after parturition of the last sow, litter sizes were equalized (CONTROL n=13.5±0.76, ADLIB n=13.7±0.89) by cross-fostering piglets. Blood samples of two heavy and light piglets per litter were taken 48h p.p to determine the immunocrit ratio (1) and γ-globulin concentration (Elphoscan-Mini Plus, Sarstedt AG & Co., Nümbrecht). On d-7 and d35 back fat thickness of each sow was measured. Differences between the groups were tested using the t-test (normal distributed) and the Wilcoxon-test (not normally distributed data; significance level: $p<0.05$).

Results: The voluntary feed intake in DM of the fibre-lactation-diet mixture a. p. was 3.14±0.68 kg. The ADLIB fed group had a significantly higher DM intake around parturition and an earlier increase in feed intake after birth (Table 1 and 2) which led to a slightly higher daily DM intake in lactation (6.97±0.39 kg vs. 7.37±0.67 kg). Birth interval between two piglets was significantly reduced in the ADLIB group (16.3^{a}±6.74 min vs. 11.0^{b}±3.49 min). A rise in salivary cortisol concentrations could be seen in both groups 24h p.p. (6.25±5.66 ng/ml vs. 5.80±4.57 ng/ml) compared to d -2 (2.27±1.54ng/ml vs. 1.52±0.93 ng/ml). While the cortisol concentration of the ADLIB group decreased thereafter, the cortisol concentration of the CONTROL group was still increasing and significantly higher 72h p.p. (7.31^{a}±6.13 ng/ml vs. 3.42^{b}±1.82 ng/ml). A significant correlation between the immunocrit ratio and the γ-globulin concentration in piglets' blood serum was found (R^2=0.81). Neither feeding regime of the sows nor piglet's birth weight influenced the immunocrit ratio (0.22±0.03 vs. 0.21±0.03) and the γ-globulin concentration (29.2±7.51 g/l vs. 27.7±7.31 g/l) which could be due to an intensive farrowing supervision. In analysed blood samples from piglets that didn't survive the lactation period (n=12) both the immunocrit ratio (0.17^{b}±0.05 vs. 0.21^{a}±0.04) and the γ-globulin concentration (19.6^{b}±7.45 vs. 28.4^{a}±9.54 g/l) were significantly lower than in surviving piglets (n=165) 48 h p.p.. The number of raised piglets (12.3±2.66 vs 12.8±1.48) and average daily litter weight gain (3.32±0.70 vs. 3.47±0.35 kg) did not differ between the groups**.** The loss of back fat thickness was in tendency lower in the ADLIB group (-3.81±1.99 mm vs. -2.39±2.15 mm).

Table 1: DM intake of sows ante partum (in kg)

Day	CONTROL	ADLIB
-4	2.63^{b}±0.23	6.49^{a}±1.76
-3	2.63^{b}±0.23	6.16^{a}±1.43
-2	2.63^{b}±0.23	5.94^{a}±1.22
-1	2.63^{b}±0.23	5.29^{a}±1.02

Table 2: DM intake of sows post partum (in kg)

Day	CONTROL	ADLIB
0	2.63^{b}±0.23	3.73^{a}±1.42
1	2.90^{b}±0.39	3.99^{a}±0.96
2	3.77^{b}±0.93	5.80^{a}±1.22
3	4.63^{b}±1.01	7.28^{a}±1.09

Conclusion: A higher feed intake during the last days a. p. did not negatively affect the farrowing process; the increased fibre intake around parturition even reduced the duration of farrowing significantly. An ad libitum feeding regime seemed to shorten stress after birth and appeared to have beneficial effects on feed intake in lactation and on mobilization of body fat reserves. The described feeding system could also be used for additional protein supplementation of high producing sows in lactation.

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105.

Influence of diet's physical form (particle size) and housing (with or without straw) on the development of gastric lesions in piglets after rearing

Einfluss des Futters und der Haltung auf die Entwicklung von Läsionen im Magen von Ferkeln nach dem Absetzen

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In the stomach of pigs the nonglandular mucosa at the entrance (PN) is mostly affected from alterations. These lesions can involve upper but also deeper layers of the stomach wall and can culminate in severe ulcerations. Although it is difficult to get an idea of the prevalence in pig herds, many investigations demonstrate that gastric lesions occur in any production stage in modern pig farming. The hypothesis of the present study was that diets' physical form and the use of straw as litter material but also "social stress" determine the risk of gastric ulcerations predominantly. After getting a status quo of gastric health at/around weaning, the influences of diet and housing (with/without litter) on the development of gastric alterations during group forming and further rearing were tested.

Methods: 4 sows and their piglets were stabled on straw. Three of the piglets were euthanized at weaning (0). 23 piglets of these litters were separated from the sow after 28 days of suckling and were grouped in a new pen, a commercial pelleted diet was fed ad libitum, and straw was used for litter. After one week (1), 5 piglets were slaughtered. Remaining piglets were randomly assigned to three different groups that were fed ad libitum a commercial diet differing in physical form, and they were kept with or without litter (straw): pelleted diet and litter (A); pelleted diet without litter (B); meal diet without litter (C). For group C the commercial pelleted diet used in groups A and B was ground after pelleting and offered as dry meal. 3 and 5 weeks after weaning each three piglets per group were slaughtered. Feed was solved in distilled water for one hour in order to analyse particle size distribution via wet sieve analysis. In all slaughtered animals stomach was removed carefully and monitored macroscopically for lesions of the PN (modified score acc. to [1]). Statistical analyses were done using the SAS software Enterprise Guide 7.1 (analysis mixed models, respectively, $p \leq 0.05$).

Results: Piglets were seen to take up straw (proved in dissection) but this parameter was not quantified. Grinding the commercial diet after pelleting resulted in an increased proportion of particles < 0.2 mm exceeding the recommendations for pig feed [2].

Group/treatment			A (+ straw)		B (- straw)		C (- straw)	
diets' particle size distribution	> 1.0 mm		32.4		32.4		24.6	
(%)[1]	< 0.2 mm		35.0		35.0		40.6	
date of slaughter (weeks after weaning)	0	1	3	5	3	5	3	5
Score[2] (x±sd)	0.0 ± 0.00	1.0 ± 2.24	0.5^{a} ± 0.50	1.5^{a} ± 2.18	3.8^{b} ± 1.30	3.8^{ab} ± 0.29	3.8^{b} ± 1.30	5.0^{b} ± 0.00

[1]wet sieve analysis [2]0=no alterations, 1-3=hyperkeratosis, 4=erosion, 5=ulceration; [a, b] indicate significant differences between the groups at the respective date of slaughter

At weaning and even after grouping with one week of fights for the hierarchy (visible external injuries) and change from milk to solid feed stomach mucosa was intact, there were no alterations apart from slight mucosal swelling (Score 1) in PN. Developing lesions on the mucosa took less than two weeks of time. The severity of gastric lesions differed between the groups: In the groups B and C minimum hyperkeratosis (Score 1-3) were present 3 and 5 weeks after weaning. No piglet in group A versus all of the piglets in group C showed ulcerative lesions 5 weeks after weaning. Compared to these results, littermates (n=12) fed a coarsely ground meal diet had a score of 0.4 ± 1.44 about eight weeks after weaning, 11 piglets had no alterations at all.

Conclusion: The effects of feed's physical form on the development of gastric alterations are well known, particle size distribution appears to be the predominant factor in this context. The appreciable uptake of straw seems to reduce the severity of gastric alterations but also seems to be accompanied with the adaptation of the piglets to the litter material [3]. While pelleted diets are and will be the mostly used form of diets for pigs, it will be important to respect particle size distribution. The addition of fibrous material to the diet might support gastric health further on.

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106.

Effects of whole wheat in the diet and different floor designs on the outcome of an experimental infection with *Salmonella* Enteritidis in broilers

Effekte von ganzem Weizen im Mischfutter und der Bodengestaltung auf den Erfolg einer experimentellen Infektion mit Salmonella Enteritidis bei Broilern

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Salmonellosis is a common zoonosis in the European Union. As main cause of *Salmonella (S.)* infection in humans the consumption of *S.* contaminated poultry meat or egg products are mentioned [1].Thus, concepts to improve food safety are needed. The hypothesis was that the provision of whole wheat in the diet and modifications in floor design (minimized contact with excreta / increase in litter quality by temperature) may reduce the spreading of an artificial infection with *S.* Enteritidis.

Methods: In two consecutive trials 240 8-day old broilers (Ross 308) were randomly assigned to 12 boxes (à 1 m²; 20 animals in each) with four different floor designs. For design I boxes were fully littered with wood shavings (1 kg/m²). For design II a floor heating was additionally installed. For design III the floor was half littered, half slatted. For design IV boxes were fully slatted and a sand bath (900 cm²) was provided. From day 10-14 of life animals were treated with enrofloxacin (10 mg/kg). On day 17 of life two birds from every box were orally infected with *S.* Enteritidis (10^8 cfu). The spread of infection was evaluated by cultural microbiological test of caecum content and liver tissue after dissection of the not-experimentally infected "contact birds" on day 23 (8 broilers p. box) and day 36/37 of life (10 broilers p. box). A conventional three-phase diet (Starter, Grower, Fattener) was offered. In the first trial 10 % of whole wheat were added after pelleting, whereas in the second trial the whole wheat was ground and included in the pellets. Statistical analyses were performed by using Wilcoxon test and Fisher's exact test for quantitative and Chi-square test for qualitative data.

Results: The table shows the microbiological results of both dissections in each trial. The lowest percentage of positive (pos.) liver samples and the lowest mean *S.* count in both trials was found in treatment II (floor heating). The partial (III) or full replacement (IV) of littered area with slatted floors showed no difference (III) or even higher rates of *S.* Enteritidis in liver and caecum content (IV) compared to treatment I. Furthermore birds fed the diet including whole wheat (Trial 1) had a considerably lower *S.* prevalence and mean *S.* counts in the caecal content compared to birds fed the pelleted diet including ground wheat (Trial 2).

	Trial 1				Trial 2			
Treatment	I	II	III	IV	I	II	III	IV
"Contact birds" (n)	53	54	54	53	54	54	54	54
Caecum, pos. (%)	39.6^{b}	29.6^{b}	46.3^{b}	67.9^{a}	92.6^{a}	100^{a}	98.1^{a}	90.7^{a}
Liver, pos. (%)	41.5^{bc}	27.8^{c}	46.3^{b}	66.0^{a}	94.4^{a}	79.6^{b}	88.9^{ab}	87.0^{ab}
Quant. tests (n)	24	24	24	24	30	30	29	28
S. counts, caecum	1.04^{ab}	0.79^{b}	1.99^{a}	1.97^{a}	4.13^{ab}	3.65^{a}	4.46^{b}	3.80^{ab}
($\log_{10}$ CFU/g)	±1.64	±1.25	±2.02	±1.85	±2.28	±1.56	±1.72	±2.06

Conclusion: The inclusion of whole wheat in the diet seems to decrease the *S.* prevalence and counts in the caecal content. Housing of birds in boxes with floor heating was accompanied with a reduced rate of translocation whereas housing in boxes with slatted floors - contrary to expectations - seems to favor the spread of infection. Indications exist that the sand bath acts like a special form of exposure.

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107.

Effect of dietary insect protein from *Tenebrio molitor L.* on lipid metabolism in an obese rat model

Einfluss von Insektenprotein von Tenebrio molitor L. auf den Lipidstoffwechsel in einem obesen Rattenmodell

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Insect protein is a novel source of protein which might become more important in the nutrition of livestock and humans in future due to the limitation of plant and animal protein resources. Studies in livestock have already shown that proteins from various insect species represent a source of protein with a high biological value. However, effects of insect protein on metabolism in animals have not yet been investigated. As the amino acid composition of insect protein shows some similarities with soy protein, a protein known for its hypolipidemic effects, we investigated the hypothesis that insect protein could beneficially influence the lipid metabolism in an obese animal model.

Methods: As an obese animal model, we used 36 male (*fa/fa*) Zucker rats which were allotted into three groups: (1) control group which received a diet with 20% casein as source of protein (C group), (2) a treatment group which received a diet in which 50% of casein was replaced on a isonitrogenous base by insect protein (I50 group), and (3) a treatment group which received a diet in which 100% of casein was replaced on a isonitrogenous base by insect protein (I100 group). The basal semisynthetic diet was composed of corn starch, saccharose, oil mixture, cellulose and a mineral and vitamin premix. As a source of insect protein, the product TMP-Y465 (Ynsect, Évry, France) isolated from ground yellow mealworms (*Tenebrio molitor L.*) was used. The product consisted of 70 % of protein and additionally contained 13 % of fat and 8 % of fibre. The amount of fat and fatty acid composition of the diets was equalized between the three groups by supplementing the diets with individual mixtures of various fats (soybean oil, rapeseed oil, linseed oil, butter) and the amount of fibre in the diets were adjusted by slightly varying the amounts of cellulose and corn starch in the individual diets. Diets were fed for four weeks. Liver and plasma samples were analysed for triacylglycerol (TAG) and cholesterol (Chol) concentrations. Moreover, a microarray analysis of hepatic gene expression was performed (n = 5/group) and metabolomics analysis of plasma samples was applied (n = 12/group). Results were analysed by one-way ANOVA and Fisher´s multiple range test.

Results: I50 and I100 rats had a higher feed intake than C rats ($P < 0.05$); final body weight was higher in I50 rats than in C rats ($P < 0.05$); final body weight of I100 rats did not differ from that of C rats. Replacement of casein by insect protein caused a dose-dependent reduction of Chol in plasma and liver and of TAG in the liver ($P < 0.05$). Metabolomics analysis revealed increased concentrations of free and acetyl carnitine, increased concentrations of phosphatidylcholine (PC) species with 2 or 3 double bonds (C34:2, C36:2, C36:3) and decreased concentrations of PC species with 4, 5 or 6 double bonds (C36:4, C38:4, C38:5, C38:6) in plasma ($P < 0.05$). The microarray analysis revealed a number of 84/224 of genes which were down-regulated and a number of 254/449 genes which were up-regulated in the liver of the I50/I100 rats in comparison to C rats (fold change ≥ 1.3 and ≤ -1.3, $P < 0.05$). Among the down-regulated genes, there were a number of genes of SREBP-1 pathway (involved in fatty acid and TAG synthesis), SREBP-2 pathway (involved in Chol synthesis pathway) and fatty acid desaturation. Relative mRNA concentration of *CYP7A1*, the key enzyme of bile acid synthesis was upregulated in the liver of I50/I100 rats compared to C rats.

Conclusion: This study shows that insect protein lowers Chol concentrations in liver and plasma and TAG concentration in liver. Data from the microarray analysis indicate that these effects might be due to a down regulation of genes involved in fatty acid, TAG and Chol synthesis and to an upregulation of genes involved in bile acid synthesis. Combination of transcriptomics and metabolomics analysis moreover shows that insect protein causes an inhibition of desaturation of linoleic- and α-linolenic acid, effects which could influence TAG and Chol metabolism in a secondary way.

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108.

Effect of species composition of grass silages on nitrogen use efficiency of dairy cows in comparison with a soybean meal and maize silage based diet

Einfluss der botanischen Zusammensetzung von Grassilage auf die N-Verwertung von Milchkühen im Vergleich mit einer Ration auf Basis Sojaextraktionsschrot und Maissilage

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Soybean meal is a common protein source in dairy cow rations. Due to its ruminally high protein stability when compared to grass or grass silages, its use results in a high nitrogen use efficiency (NUE) compared to protein from grassland. To overcome the constraints of sustainability regarding the production and use of soybean meal, grassland-based milk production is promoted in Switzerland. This requires forages which result in a high NUE. The use of feeds with elevated contents of plant secondary metabolites (PSM) is a possible approach to improve the NUE in grass based rations. Forbs, such as plantain (*Plantago lanceolata* L.) contain more PSM compared to grass. The aim of the present study was to compare three diets with different species composition (grass alone, +legumes, +herbs) compared to a reference diet based on maize silage and soybean meal for milk production and quality.

Methods: Silages were produced from three different leys based on a rye grass monoculture (G), a grass-clover mixture without (GC) and with plantain (*P. lanceolata*) (GCP) in 2015. The botanical composition (% DM) at harvest was as follows: G: *Lolium perenne* (100%); GC: *L. perenne* (67.8%), *Trifolium pratense* (25.7%), *Trifolium repens* (4.4%), other (2.1%); GCP: *L. perenne* (40.9%), *P. lanceolata* (40.8%), *T. pratense* (14.8%), *T. repens* (2.7%), other (0.8%). The three test diets (G, GC, GCP) and a reference diet (M) balanced for energy (NEL) and metabolisable protein (MP) were fed for *ad libitum intake.* Diet M was composed of maize silage, soybean meal and hay. The diets were supplemented with 3 kg of wheat per cow and day. Each diet was fed to six lactating dairy cows (n=24) balanced for milk yield (39.3 ± 5.6 kg energy corrected milk (ECM), 1.4 ± 0.2 kg fat, 1.2 ± 0.2 kg protein), days in lactation (171 ± 68), number of lactations (3.5 ± 1.2), breed (Brown Swiss n=8, Holstein n=16) and body weight (645 ± 56 kg). After 13 days of adaptation, during 7 days dry matter intake, milk yield and milk composition (Suisselab AG, Zollikofen) were measured. Statistical analysis was performed using R (V. 3.2.3, 2015) with diet as fixed effect and period (3 × 2 cows per treatment) as block factor. Tukey's test was used for multiple comparisons among means.

Results: The crude protein (CP) contents (% of dry matter (DM)) of the diets as consumed were 14.7% (M), 12.8% (G), 16.3% (GC) and 17.4% (GCP) respectively. Intakes of DM and CP were 19.0 and 2.8 for M, 14.5 and 1.9 for G, 17.4 and 2.8 for GC, and 20.7 and 3.6 kg d^{-1} for GCP. Milk yield (kg ECM d^{-1}) did not differ for M (27.9), GC (24.1) and GCP (27.4) but was significantly lower (P<0.05) for G (20.6). Milk protein yield (kg d-1) was similar for M (0.91) and GCP (0.91) but significantly lower (P<0.05) for G (0.68) and GC (0.74). The results for NUE (g milk protein N kg N intake-1) were 0.33 (M), 0.37 (G), 0.26 (GC) and 0.25 (GCP) respectively. Milk urea yield (g d-1) was significantly higher (P<0.05) for GCP (8.1), as for M (5.9) and GC (5.3), and lower (P<0.05) for G (3.1).

Conclusion: The dietary CP content and, with that, milk urea amount secreted, increased from G to GC to GCP. Across all diets, a positive linear relationship (R^2=0.98) between milk urea excretion and CP intake (g/day and cow) was measured. The results indicate that only G with the limiting CP content was able to yield a higher NUE than the reference diet with soybean meal as a protein source.

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109.

Comparative evaluation of lucerne, grass and maize silage-based rations for lactating dairy cows: Feed intake, milk yield and milk composition

Vergleichende Bewertung von Luzerne-, Gras- und Maissilage basierten Rationen für Milchkühe: Futteraufnahme, Milchleistung und Milchzusammensetzung

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Lucerne (*Medicago sativa L.*) is more efficient at using water than other forage species and is therefore suitable for cultivation in spring- or summer-dry regions; there has been a revival of interest in its use in the light of climate change. The objectives of this study were to measure digestibility by sheep of grass, maize and lucerne silages and to evaluate dry matter (DM) intake (DMI), milk production and milk composition for lactating dairy cows fed diets based on the three silage silage types in different combinations.

Methods: Lucerne, grass and whole-crop maize silages were offered to lactating dairy cows in various combinations and feed intake, milk yield and milk composition were determined. Cows were fed four different total mixed rations (TMR) with forage to concentrate ratios of 55:45 (dry matter (DM) basis). The forage component of the control (CON) ration was a 50:50 mixture of grass and maize silages (DM basis). The forage component of the three lucerne-based diets comprised (DM basis) either grass and lucerne silage (50:50; GL), maize and lucerne silage (50:50; ML) or equal proportions of the three silage types (GML). The TMR were formulated to have the same protein content, expressed as utilisable crude protein at the duodenum (uCP) but rations differed with regard to energy, fibre and starch concentrations and structural value. A seven-phase feeding trial was conducted with a herd of 60 lactating cows. Each diet was given to the whole herd for a period of six weeks. After six weeks the diet was changed to the next treatment over 3 to 4 days. During phases 1, 3, 5 and 7 the cows were fed with the CON diet. In phases 2, 4 and 6, respectively, the GL, ML and GML diets were offered. All lactating cows received the diets in the same order over the same period. Performance data from the feeding trial with dairy cows were analysed using the mixed model procedure in SAS with the maximum likelihood method. Treatment effects were evaluated by comparing phases with lucerne TMR with the average of the preceding and subsequent phases in which no lucerne was fed.

Results: In the digestibility study on sheep, lucerne silage was found to be less digestible in terms of organic matter and gross energy than grass or maize silages; the fibre fractions were also less digestible than those of grass silage. In the dairy cow trial, daily DMI ranged from 20.8 kg to 22.3 kg and was greater ($P<0.05$) when cows were fed the lucerne silage diets. Cows were only able to compensate for the lower energy density of the lucerne diets when they were fed the combination of maize and lucerne silages; the greater DMI of the maize and lucerne diets relative to the CON diet allowed them to achieve the same net energy intake per day. The average energy-corrected milk yield ranged from 31.7 and 33.6 kg/day across treatments. Including lucerne silage in the TMR reduced milk yield, milk protein concentration and milk protein yield. Only the combination of lucerne and grass silage increased milk fat content relative to the CON diet (4.31 vs. 3.89%).

Conclusion: Lucerne silage in TMR for lactating dairy cows increased DMI but decreased milk yield compared with diets whose forage portion consisted of grass or maize silages. The aim should be to harvest lucerne earlier in order to achieve a higher crude protein content, less lignification and hence higher nutrient digestibility. Improving the protein value and digestibility of lucerne forage would further reduce the need to include soybean meal and rapeseed meal in diets including lucerne, relative to a control diet based on maize and grass silages.

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110.

Search for European plants containing bioactive compounds to lower *in vitro* methane formation from ruminally fermentable organic matter

Suche nach wirkstoffhaltigen europäischen Pflanzen zur Verringerung der in vitro Methanbildung aus pansenfermentierbarer organischer Masse

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To decrease the methane formation in ruminants, studies aim at specific feeding strategies. Secondary plant compounds might play a central role for this purpose. Achieving a reduction of methane production by 15-25% without negatively affecting digestibility has already been demonstrated for selected plants in previous studies. The purpose of the present study was to test plants containing secondary compounds like phenols, which grow naturally in Europe. We compared different types of plant material obtained from 16 plant species for their ability of methane mitigation and their feeding value in an *in vitro* experiment.

Methods: The following materials were tested: Leaves from *Arctostaphylus uva-ursi, Betula pendula, Castanea sativa, Corylus avellana, Populus tremula, Ribes nigrum, Salix caprea as well as* green and red *Vitis vinifera*; fruits from *Aesculus hippocastanum and Prunus spinosa*; the root from *Paeonia alba* and the above ground part from *Epilobium angustifolium, Geum urbanum, Symphitum officinale* and *Lotus corniculatus*. Thirteen of these plants have been tested in the completed "Rumen up" project (http://www.abdn.ac.uk/research/rumen-up/report/) and were shown to mitigate *in vitro* methane production by more than 15%. Three further plants were selected due to their known benefits for ruminant performance. The content of crude protein and neutral detergent fiber in the material ranged from 2 to 17 % and 16 to 69%, respectively. The plant material contained 0.5 to 27% total phenols and 0.2 to 18% total tannins. Two independent batches from each additive were tested in six independent runs with the Hohenheim Gas Test method (1). Incubation was performed with 40 mg of test plant material added on top of 200 mg of a basal diet (silage:hay:concentrate, 75:10:15 %) and incubated with a rumen buffer mixture (1:2) for 24 h. Diets containing 200 g/kg of well palatable plant leaves were found repeatedly to be consumed without suppressing intake. Total gas, methane and carbon dioxide production as well as pH and ammonia concentration were measured after incubation. *In vitro* organic matter digestibility (IVOMD) and NEL contents were calculated according to Menke and Steingass (1). Values from the basal diet without plant additive were used as control. The data were analyzed with the mixed procedure in SAS with a Tukey-Kramer adjustment; differences among means were considered to be significant at $p<0.05$.

Results: The pH ranged between 6.7 and 6.9 and, compared with the basal diet, the values were lower with most additions of plant material due to the extra fermentable matter. Ammonia concentrations decreased ($P < 0.001$) by up to 20% when adding most of the test materials, which is considered to be advantageous. Only in five out of the 18 test materials IVOMD significantly declined and four of them (*C. sativa, E. angustifolium, P. tremula* and *P. spinosa*) thereby significantly reduced the estimated NEL content of the complete diets.. Methane per unit of digestible organic matter was 50.4 ml/g in the control and was lowered ($P < 0.001$) by thirteen plant additives in an extent of 5 to 20%. Methane yield per unit of feed dry matter showed similar variations.

Conclusion: Although the methane mitigating effect of the plants was occasionally less pronounced than that reported earlier, the leaves of chestnut (*C. sativa*), fireweed (*E. angustifolium*) and hazelnut (*C. avellana*) were particularly promising in terms of methane mitigation. When added to a high quality basal diet, most test plants did not depress *in vitro* digestibility and NEL content indicating that their forage value is basically favourable. This is important for a future strategic implementation in ruminant diets. In a subsequent *in vivo* experiment with dairy cows, the effect of the most promising plant additives on feed intake and their methane mitigation potential will be examined.

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111.

Effect of brown seaweed *Ascophyllum nodosum* on rumen fermentation and nutrient digestibility in sheep

Einfluss von braunem Seegrass Ascophyllum nodosum auf Pansenfermentation und Nährstoff Verdaulichkeit von Schafen

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Introduction: Brown seaweed (SW) is known for its high content of minerals (esp. iodine) but it also contains unique phenolic compounds like phlorotannins. Phlorotannins are described to have antibacterial, antiviral, and antifungal properties which raises the interest of using SW as supplement for functional food and livestock feed (1). Tasco-AOS meal (Arcadian Seaplants Ltd., Dartmouth, Canada) is a commercial SW product (8.0% crude protein (CP), 1.8% crude fat, 2.2 % phlorotannins; dry matter (DM) basis) produced from dried, ground *Ascophyllum nodosum* of the Fucaceae family. The objective of this study was to determine the effect of titrated levels of SW from Tasco-AOS meal on rumen fermentation and nutrient digestibility in sheep.

Methods: Eight ruminally cannulated Arcott rams (63.3 ± 4.1 kg initial body weight) were used in a replicated 4 × 4 Latin square (4 diets × four 21-d periods). The control diet (0% SW) consisted of 48.4% barley grain, 35.9% dehydrated alfalfa, 6.7% beet pulp, 4.0% mixed hay, 2.4% beet molasses, and 2.6% mineral vitamin premix (DM basis). Seaweed diets were formulated by replacing 1.0, 3.0, and 5.0% of barley in the control diet with Tasco-AOS meal. Apparent total tract digestibility was measured between d 18 and 21. Ruminal fermentation measurements [NH_3, volatile fatty acids (VFA), and protozoa counts] were taken 0, 3, 6, and 12 h after feeding on d 21. Data were analysed using the MIXED procedure of SAS. The model included the fixed effects of diet and the random effects of square, ram within square, and period within square. Contrasts were used to determine linear and quadratic responses to the dietary SW concentrations. Treatment effects were declared significant at $P < 0.05$.

Results: Apparent total tract digestibility of CP decreased linearly ($P < 0.01$) with increasing concentration of SW. Digestibility of DM, organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) was not affected by SW ($P \geq 0.10$). Concentration of acetate increased linearly ($P < 0.001$), while propionate (both mol/100 mol) decreased linearly in response to increasing level of SW ($P = 0.046$). Feeding SW resulted in a linear increase of rumen protozoa numbers ($P < 0.01$) and higher concentration of rumen NH_3 compared to the control ($P < 0.01$).

Table 1. Nutrient digestibility and ruminal fermentation variables of rams (n=8) fed 0, 1, 3 and 5% seaweed (SW; DM basis).

	Diet				P-value		
	0% SW	1% SW	3% SW	5% SW	CON vs. SW	Linear	Quadratic
Digestibility,%							
DM	76.1	75.0	73.9	71.3	0.104	-	-
OM	78.2	77.5	76.2	73.9	0.106	-	-
CP	70.9	69.9	67.6	64.0	0.064		0.693
NDF	51.7	50.6	52.3	46.5	0.560	-	-
ADF	40.6	42.3	42.0	36.9	0.942	-	-
Total VFA, mM	142	155	154	144	0.172	-	-
VFA, mol/100 mol							
Acetate	50.2	56.7	55.1	57.8			0.09
Propionate	27.9	21.5	27.1	22.8		0.046	0.97
Butyrate	18.3	18.0	14.5	15.9	0.007		0.03
NH_3, mM	6.47	12.50	8.50	10.59		0.159	0.24
Protozoa, n × 10^6/mL	2.18	6.30	4.82	8.09			0.06

Conclusion: Apart from a decrease in CP digestibility, supplementing SW had no adverse effect on total tract digestibility. Numerical declines in DM and OM digestibility were most likely due to the reduction in CP digestibility. Changes in rumen fermentation, like the increase in protozoa numbers and NH_3 concentration in response to SW were profound and indicate that SW modulates ruminal microbial structure. Investigations on a molecular level are under way.

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112.

Use of nylon bags during *in vitro* fermentation: Effects on gas production, feedstuffs degradability and short-chain fatty acids concentration

Verwendung von Nylonbeuteln zur in-vitro-Inkubation von Futtermitteln: Auswirkungen auf die Gasbildung, den Nährstoffabbau und die Bildung kurzkettiger Fettsäuren

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In vitro rumen fermentation techniques are very useful tools to screen across a large number of samples without the need for *in vivo* trials. However, in cases where mixed substrates are incubated (e.g. concentrate + forage) it is not possible to separately do further analyses (e.g. degradability, starch content) in each residual fraction of the substrate after the *in vitro* incubation. Therefore, in this study the use of nylon bags (pore size = 50 µm) inside the *in vitro* incubation unit was studied for its effects on fermentation parameters using the ANKOM RF technique.

Methods: Two forages, maize silage and grass hay, were incubated in separated flasks (Duran bottle, 500 ml) along with concentrate in a 70:30 ratio in duplicate in three different runs each. Rumen fluid was collected from two fistulated Jersey cows before morning feeding and mixed with a bicarbonate buffer in a 1:1 ratio. Gas production (GP), true dry matter digestibility (TDMD), and short chain fatty acid (SCFA) concentrations were measured. Three experiments were performed. Experiment (I), 2 g of sample were incubated with 300 ml of inoculum during 48 h. Three treatments were included:Control (substrate freely incubated),Forage.In (the forage part was incubated inside the nylon bag and concentrate was set freely), andConc.In (the concentrate was incubated inside the nylon bag, and the forage was set freely). Experiment (II), the sample size was reduced to 1.2 g to increase the bag:sample ratio (1) and the incubation time was 24 h. The Control andConc.In were considered in this experiment. Experiment (III) was performed following a similar procedure from Experiment (2), but nylon bags were washed with acetone prior to the incubation, as surfactants in the bag may inhibit microbial activity (2).

Results: In the first experiment both Forage.In and Conc.In decreased GP, TDMD, and total SCFA yield ($P < 0.01$) after 48 hours, indicating an impairment of the fermentation. The decrease in these parameters was greater for Forage.In than for Conc.In, thus the former was excluded from further experiments. Decreasing the bag:sample ratio minimized the differences between Control and Conc.In where GP decreased (Experiment II). Washing the bags with acetone (Experiment III) did not further minimize the differences between Control and Conc.In with a decrease in SCFA. Regression across the measured parameters of Control on Conc.In showed R-Square values of 0.86, 0.95 and 0.97 for SCFA, TDDM and GP, where incubating the concentrate inside the bag (Conc.In) always underestimated these parameters compared with the Control.

Table 1. Gas production, true dry matter degradability (TDMD) and short chain fatty acids (SCFA) concentration when the forage (Forage.In) or concentrate (Conc.In) were incubated inside nylon bags.

Variables	Forage [1]	Experiment I (48 h)			Experiment II (24 h)		Experiment III (24 h)	
		Control	Forage.In	Conc.In	Control	Conc.In	Control	Conc.In
Gas production (ml/g DM)	MS	260^{a}	236^{c}	251^{b}	216^{a}	193^{b}	185	179
	GH	187^{a}	176^{c}	179^{b}	123	118	123	117
TDMD (g/100 g)	MS	89.5^{a}	87.4^{b}	88.8^{a}	79.2	80.0	77.4	77.1
	GH	81.6^{a}	76.4^{b}	80.0^{a}	66.1	68.4	73.1	72.6
SCFA (µM/ml)	MS	36.7^{a}	31.6^{b}	32.7^{b}	29.1	28.6	24.8^{a}	23.0^{b}
	GH	25.3^{a}	21.7^{b}	22.6^{b}	19.3	17.9	16.5	15.1

[1] MS = Maize silage; GH = Grass hay

Different superscript in rows within an experiment show differences between means ($P < 0.05$)

Conclusions: Results suggest that incubating forage inside a nylon bag greatly impairs fermentation of this substrate, while lesser effects appear when the concentrate portion is incubated inside the bag. The micro-environment inside the nylon bag, due to accumulation of fermentation products, may inhibit the activity of microorganisms. This effect was minimized by reducing the bag to sample size ratio and washing bags with acetone. Nylon bags can be used in combination with an *in vitro* fermentation system as long as all samples are incubated under the same conditions. However, some evidence was found of a bag by forage interaction that requires further attention.

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113.

Effect of a high fiber diet before and during lactation on sow and offspring performance, fecal characteristics and the relation to neonatal *Clostridium difficile* colonization

Einfluss eine faserreichen Diät vor und während der Laktation auf die Leistung von Sauen und deren Nachkommen, Fäzescharakteristika und der Bezug zur neonatalen Besiedlung mit Clostridium difficile

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Question: Despite legislative aspects regarding a required minimum dietary crude fibre content for animal welfare reasons in pigs (i.e. in gestating sows, organic farmed pigs), increasing the dietary fiber content in lactating sows could be an attractive approach to manipulate performance and health in the offspring. Intestinal health in neonatal piglets is influenced by many factors including milk composition and intake, early life microbial environment and colonization by putative pathogens such as *Clostridium difficile* (CD) strains. In the current study, we hypothesized that a high fiber diet during gestation and lactation will modulate sow and piglet performance due to maternal energy balance, and will also affect intestinal ecology and early-life colonization of piglets with CD.

Methods: Pregnant sows in the control group (CON, n=8) were fed a standard gestating (per kg DM: 11.9 MJ ME, 16.0% CP, 7.5% CF) and a standard lactating diet (13.0 MJ ME, 17.9% CP, 5.0% CF) after farrowing. Sows in the high fiber group (HF, n=8) received the same diets but with the addition of 10% of dried whole crop rye meal (6.8% ash, 14.2% CP, 33.7% CF) yielding higher CF (10.1 and 7.9% for gestating and lactating diets, respectively) and lower energy concentrations (10.8 and 11.9 MJ ME, respectively) in the diets. Feed intake of sows during lactation was recorded daily. Body weight of sows and piglets was recorded weekly and energy balance of sows was calculated using feed intake data and milk energy output as calculated from litter gain. Fecal samples were taken one week prior (sows, n=8) and two weeks after farrowing (sows and piglets) for pH and short chain fatty acid analysis. Milk samples were analyzed for nutrient composition. The concentration of CD was determined by quantitative real-time PCR. Data were analysed by Mann-Whitney U test. The statistical significance was considered at $P<0.05$.

Results: Total number of live born (13.8 vs. 14.3 for CON and HF, respectively) and weaned piglets (13.1 vs. 12.8 for CON and HF, respectively) per sow did not differ ($P>0.05$). However, average birth weights of piglets tended to be higher in CON sows compared with HF sows (1.43±0.31 kg vs. 1.34±0.30 kg, $P<0.1$). In contrast, piglets from HF sows tended to be heavier than from CON sows (6.92±1.42 kg vs. 6.56±1.14 kg, $P<0.1$) at weaning and had a significantly higher daily weight gain during the suckling period (227±49 vs. 208±41 g, $P<0.05$). Total feed intake of sows fed CON and HF diets did not differ, but the energy balance was lower in HF sows. Accordingly, HF sows had higher body weight loss as compared to CON sows (16±8 vs. 9±5, $P<0.1$) during lactation and higher milk fat content ($P<0.05$). Sows fed HF diets had lower fecal total SCFA concentrations (88±19 vs. 109±18; $P<0.05$), which was mainly due to lower propionate and butyrate levels, and higher fecal pH (6.4±0.4 vs. 6.1±0.2; $P<0.1$) as compared to sows fed CON diets. Similarly, fecal total SCFA concentration was lower (30±7 vs. 38±11; $P<0.05$) and pH was higher (7.4±0.4 vs. 7.1±0.4, $P<0.05$) in piglets of sows fed HF diets as compared to CON sows, suggesting indirect effects of sow diet on offspring intestinal microbial composition and/or activity. Finally, piglets from sows fed HF diets had a higher colonization with CD (5.68±0.66 vs 5.6±0.84 log 16S gene copy numbers, $P<0.1$).

Conclusions: Taken together the data suggest differences in energy metabolism in sows fed high or normal levels of crude fiber during gestation and lactation. Reasons are not clear but might be due to metabolic adaptation or differences in hindgut fiber fermentation. The data also demonstrate that high fiber diets for sows affect the colonization of piglets with CD. Since in the present study, a rather insoluble fiber source was used, further studies should also focus on the effects of more soluble (and higher fermentable) types of fiber and the factors favoring CD colonization in piglets.

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114.

Roughage based liquid diets for pregnant sows - apparent digestibility of whole plant silages (wheat, rye) and their nutritive value

Grundfutter-basiertes Flüssigfutter für tragende Sauen - scheinbare Verdaulichkeit und Futterwert von Ganzpflanzensilagen (Weizen, Roggen)

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Some decades ago it was quite common to feed pregnant sows grass, beets and silages as being cheap sources for energy and nutrients compared to concentrates. Traditionally "combined feeding" was used, but in modern production units this concept has lost its previous relevance. In large units it is necessary to have roughages which can be offered with low input of manual work, and without resulting in failure of the manure system. With modern techniques of diminution it is nowadays possible to use WPS as a basis for liquid diets including further ingredients and supplements. Also it is easy to vary the energy and nutrient density in the liquid diet by changing the proportion of silage to concentrate. Thus the nutritive value of silages is again on the agenda, last but not least stimulated by diverse efforts on animal welfare (i.e. prevention of ethopathies due to restrictive feeding concepts). Hypothesis of this experimental study was that liquid diets based on silages can be offered ad libitum without risk for luxury feed consumption and overfed sows.

Methods: Four gestating sows (bw: 200-240 kg) were kept individually and fed a ration consisting of conventional pelleted concentrate mixed with one whole plant silage per trial: whole plant wheat silage (WPWS) - coarsely ground (c.g.), WPWS - finely ground (f.g.) and whole plant rye silage (WPRS) - f.g. Ten days of adaptation to the diet were followed by a five day period of collecting refusals and faeces completely to calculate the aD of the WPS via difference method (aD of the compound feed analyzed before).

Results: The aD of the three WPS differed (WPWS>WPRS) depending on the XF level; the calculated values of the aD_{OM} fitted quite well to expected values when the formula regression of [1] is used. It has to be underlined that aD of crude fiber was low (~19-27%), resulting in markedly increased masses and volumes of faeces. The sows showed a preference to the ration when the WPS was offered mixed with the concentrate and water (25% DM of the whole ration). The average daily intake resulted in an XF intake of 13.0% (WPWS, c.g)/ 13.7% (WPWS, f.g.)/ 17.0% (WPRS, f.g.) of DM of the whole ration, exceeding values of at least 8 % of DM, set by legislation [3].

Table 1: Apparent digestibility (aD) of whole plant silages in comparison in pregnant sows

WPS, n=4	WPWS, c.g.	WPWS, f.g.	WPRS, f.g.
DM content, g/kg as fed	363	425	492
XF content, g/kg DM	229	214	285
DM intake/d, kg[1)]	0.868 ± 0.001	0.981 ± 0.079	1.21 ± 0.036
aD OM expected, % [1]	53.7	56.2	44.3
aD OM, %	54.1 ± 3.18	60.0 ± 2.33	46.8 ± 0.843
aD XP, %	53.2 ± 7.15	59.9 ± 4.21	45.0 ± 8.84
aD XL, %	67.0 ± 10.5	46.4 ± 9.43	28.3 ± 9.38
aD XF, %	18.8 ± 4.50	24.2 ± 4.97	27.0 ± 4.74
aD NFE, %	66.8 ± 2.28	72.3 ± 2.19	58.6 ± 4.69
Energy[2)], MJ ME/kg DM	8.88	9.72	6.77

[1)] aD-trial (5d) [2)] calculated by equation 3 [2]

Conclusion: During the digestibility trial the roughage intake reached 0.41-0.55% of the sows' body weight, additionally to the intake of compound feed. In the complete liquid diet 30-37% of the energy derived from WPS. Regarding energy supply, diets of higher crude fiber content may prolong the satiation, which should favor wellbeing. Offering 1.2 kg DM compound feed and silages additionally, an individual daily WPS intake of 1.5 ± 0.420 (WPWS, f.g.) or 1.3 ± 0.227 (WPRS, f.g.) kg DM was achieved. Although the silages were available to free access, the intake was limited, i.e. there is a chance to feed WPS based liquid diets ad libitum without any risk for an excessive feed and / or energy intake.

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115.

Variation in water holding and linear buffering capacity of fiber rich feed stuffs

Wasserbindungskapazität und lineare Pufferkapazität verschiedener faserreicher Futtermittel

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Dietary fibre is physiologically the part of carbohydrates which is not digested by enzymes of the intestine but partly fermented by the intestinal microflora, especially in the hindgut (of monogastric species) (1). Because of that it represents the part of the feed or ration that crosses nearly the whole gastrointestinal tract (GIT). Thereby physicochemical properties like water holding capacity (WHC) and linear buffering capacity (LBR) of dietary fibre can affect the digestive processes. Knowing such properties supports diets formulation e.g. for piglets with limited amounts of HCl production and higher susceptibility to diarrhoea. The objective was to determine the water holding capacity and linear buffering capacity of a range of fibre rich feeds varying in soluble and insoluble fibre content. Establishment of practicable *in vitro* methods was the precondition for the outcome ranking.

Methods: The study included 22 verities of hulls, brans, lignocelluloses and other fibre rich by-products. The measurement of LBR was performed as described in Braach *et al.* (2) by stepwise pH lowering from pH 8 to pH 2 with HCl. WHC was established comparing two main methods: 1) with 0.25 to 0.5 g of whole sample (≤ 1mm), 24h presoaking in 10 ml distilled water and finished by centrifugation (15 min, 5000 rpm); 2) by soaking 0.25 to 0.5 g whole sample (≤ 1mm) with 10 ml distilled water (24h) and stirring (first 2h) in cylinder with no external fore influence. Supernatant was assumed as the non-absorbed water and was removed, the amount in ml noted and used to calculate the WHC for both methods [gH_2O/gDM = ((ml H_2O / 1000) / initial DM weight) *1000]. For method 2 the swelling properties (SwP) were expressed in percentage as difference between the starting volume and the final volume obtained after 24 h. Further analyses are shown in table 1: crude fibre (CF), neutral detergent fibre ($aNDF_{OM}$), acid detergent fibre (ADF_{OM}), acid detergent lignin (ADL_{OM}), soluble, insoluble and total dietary fibre (SDF, IDF and TDF) as well as crude ash (CA), crude protein (CP).

Results: WHC was on average 6.22 (WHC1), respectively 5.06 (WHC2) gH_2O/DM. The highest value was observed for beet pulp (18.55 / 16.58) and the lowest for sorghum bran (2.90 / 2.27). SwP was for half of the feeds less than 100% with maximum swelling in beet pulp (963%) and minimum in hemp and sorghum bran (33%). For LBR the mean value was 4.67 with rape hulls with the highest (6.58) and lignocellulose II (2.47) with the lowest value. The fibre contents showed the wide range of fibre rich feeds. TDF values varied between 41-100%, $aNDF_{OM}$ from 30-93%, CF from 14-58% and CP content was less than 18%.

Table 1: WHC (g H_2O/ g DM), SwP (%), LBR and relevant ingredients (g) of fibre rich feeds.

feeds	WHC1	WHC2	SwP	LBR	CF	$aNDF_{OM}$	ADF_{OM}	ADL_{OM}	SDF	IDF	TDF	CA	CP
beet pulp	18.55	16.58	963	5.08	153	317	196	87	163	474	637	127	100
apple pomace	11.37	8.83	550	3.69	234	476	368	246	146	530	676	20	69
wheat straw	7.80	7.43	263	3.55	396	826	508	218	15	838	853	52	30
lignocellulose I	7.29	5.68	236	2.47	579	926	728	650	11	942	953	5	8
soybean hulls II	6.40	5.55	205	5.18	301	562	388	70	70	585	654	52	179
lignocellulose III	6.35	5.51	185	4.14	561	874	737	335	12	938	949	7	10
pectin	6.33	5.34	178	6.33	174	n.a.	n.a.	n.a.	311	416	726	27	52
lignocellulose II	6.30	5.21	150	2.44	559	919	757	329	13	933	945	10	8
rape hulls	6.25	5.19	145	6.58	334	622	529	363	23	578	601	50	169
lupine hulls	5.93	5.10	135	5.64	559	870	715	27	27	942	968	20	42
sunflower hulls	5.88	4.63	96	4.57	535	843	679	255	27	871	897	26	41
soybean hulls I	5.60	4.48	70	4.68	375	687	507	29	77	713	789	49	113
spelt hulls I	5.51	4.38	70	4.51	398	856	494	86	6	866	872	60	18
wheat bran	5.09	4.35	67	5.74	145	585	181	70	34	579	612	75	159
spelt hulls II	5.03	4.31	67	2.69	384	803	477	114	11	830	841	57	31
grape pomace, white	4.42	3.82	65	5.24	203	298	292	237	50	363	413	47	90
vinasse, dried *	4.28	3.35	52	4.37	338	818	565	298	14	749	763	7	168
grape pomace, red	3.52	3.07	44	6.38	318	455	386	359	42	558	600	58	131
rice bran	2.92	3.04	40	4.77	460	794	607	249	0	785	785	179	19
hemp bran	2.91	3.02	33	4.89	397	660	506	231	n.a.	n.a.	n.a.	33	175
sorghum bran	2.90	2.27	33	5.09	430	810	574	208	6	804	810	127	40
cellulose, pure	n.a.	0.22	15	4.79	n.a.	n.a.	n.a.	n.a.	0	1000	1000	n.a.	5

* fermentation residues, n.a.: not analysable.

Conclusion: The obtained data show the variety of WHC and LBR of fibre rich feeds, which may influence their choice in diet formulation.

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116.

Investigations on vitamin B_2 and B_6 contents in organically produced cereal and grain legume varieties

Untersuchungen zum Gehalt an Vitamin B_2 und B_6 in ökologisch angebauten Getreide- und Körnerleguminosensorten

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Diets deficient in vitamin B_2 (B_2) and vitamin B_6 (B_6) can largely affect animal health. Thus, those vitamins are commonly supplemented even in organic farming. However, information on actual contents of B_2 and B_6 in different feedstuffs is scarce. Therefore, we analyzed some cereals and grain legumes and investigated the differences in B_2 and B_6 content between varieties.

Methods: In three years (2011 - 2013), samples of wheat (*Triticum aestivum* L., winter n=106, spring n=45), barley (*Hordeum vulgare* L., winter n=30 in two years, spring n=81), winter rye (*Secale cereale* L., n=106), winter triticale (*Triticosecale* L., n=107), oats (*Avena sativa* L., n=105), blue lupins (*Lupinus angustifolius* L., n=87), spring field peas (*Pisum sativum* L., n=87), and spring field beans (*Vicia faba* L., n=77) were collected from organic variety trials in Germany. The samples were dried at 40 °C and ground to pass a 0.5 mm sieve. They were analyzed for their contents of B_2 and B_6 using a modified HPLC-method with FLD detection (1).

Mixed procedures with subsequent Tukey-Kramer-tests ($\alpha = 0.05$) were used (proc MIXED, SAS 9.4) to create lsmeans for B_2 and B_6 in the cultivars (year and cultivar*year as random effects) and find differences between varieties of a cultivar. Only varieties with more than three repetitions were considered for variety comparisons. Harvest year, cultivation site, and all interactions were assigned as random effects. Model optimization was conducted using the bayesian information criterion (BIC).

Results: Cultivars: There were similar B_2 contents in different cereal cultivars. Oats had the lowest mean B_6 content (Table 1). In grain legumes, beans had highest contents of B_2 and B_6 (2.75 ± 0.06 and 1.63 ± 0.09 mg kg^{-1} DM). Lupins and peas contained 2.36 ± 0.06 and 1.73 ± 0.06 mg B_2 as well as 0.52 ± 0.09 and 0.74 ± 0.09 mg B_6 kg^{-1} DM, respectively. All cultivars contained lower amounts of B_2 and B_6 compared to literature results (2, 3). This might be due to agricultural management, different analytical methods, sample preparation, as well as duration and conditions of storage.

Table 1: Contents of B_2 and B_6 in the observed cereal cultivars (LSMean and SE in mg kg^{-1} DM)

	Winter wheat	Spring wheat	Winter barley	Spring barley	Winter triticale	Winter rye	Oats
n	106	45	30	81	107	106	105
B_2	0.75 ± 0.06	0.84 ± 0.06	0.82 ± 0.08	0.94 ± 0.06	0.91 ± 0.06	1.06 ± 0.06	1.00 ± 0.06
B_6	1.12 ± 0.09	1.37 ± 0.10	1.64 ± 0.11	1.12 ± 0.09	0.97 ± 0.09	0.59 ± 0.09	0.29 ± 0.09

Varieties: Differences in varieties were mostly rather low in cereals. The highest significant differences in B_2 were found between spring wheat varieties (0.23 mg kg^{-1} DM). Winter triticale varieties had the highest significant differences (max. 0.28 mg kg^{-1} DM, $p < 0.05$) of B_6. However, there were no significant differences in B_2 and B_6 between three winter barley varieties. Furthermore, B_6 did not differ between three varieties of winter wheat and was equal in seven oat varieties ($p > 0.05$). In lupins, of five varieties Boruta had the lowest and Probor the highest B_6 content (0.32 ± 0.09 and 0.65 ± 0.09 mg kg^{-1} DM, $p < 0.05$). There were high significant variations in B_6 between seven pea (KWS La Manscha 0.25 ± 0.09 and Auckland 1.37 ± 0.11 mg kg^{-1} DM) and eight bean (Julia 1.26 ± 0.17, Espresso 2.23 ± 0.18 mg kg^{-1} DM) varieties. B_2 did not differ between five varieties of lupins. However, the B_2 content varied significantly between pea (Navarro 1.65 ± 0.09 and Salamanca 1.84 ± 0.09 mg kg^{-1} DM) and bean (Divine 2.35 ± 0.05 and Alexia 3.35 ± 0.06 mg kg^{-1} DM) varieties.

Conclusions: The results indicate that the contents of B_2 and B_6 can possibly be influenced by genetic factors. However, the potential might be higher in grain legumes than in cereal grains. Investigations on B-vitamin contents in further feedstuffs like alfalfa, clover, or natural feed supplements are needed to re-evaluate the need of B-vitamin supplementation in organic diets.

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117.

Effect of rumen nitrogen balance on *in vitro* gas production and microbial protein synthesis from different carbohydrate sources

Effekte der ruminalen Stickstoffbilanz auf die in vitro Gasbildung und mikrobielle Proteinsynthese bei unterschiedlichen Kohlenhydratquellen

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Minimization of nitrogen emissions by reducing nitrogen concentrations in diets has been a research focus over decades. Asynchronous/inadequate nitrogen and energy may affect the ruminal fermentation and microbial growth in dependency with the sources of energy i.e. carbohydrate sources. Hence, an *in vitro* study on the effects of carbohydrate sources differing in rate and extend of degradation, with different levels of nitrogen supply on the fermentation and microbial protein synthesis was performed.

Methods: Three carbohydrate sources (i.e., sucrose, corn starch, cellulose) were incubated at three rumen nitrogen balance (RNB) levels (i.e., 0, -5, g/kg dry matter) using grass hay as forage and urea as single source of nitrogen. In total, two grams (as-fed basis) of a substrate mixture were incubated with 200 ml of McDougall's buffer (1) and 100 ml rumefluid during 24 h in an *in vitro* ANKOM-RF system. One gram (as-fed basis) of grass hay was weighed per flask, whereas the proportion of urea and carbohydrate source changed to achieve the target RNB. Actual RNB was determined after incubation (2). Gas production (GP), concentrations of short-chain fatty acids (SCFA) and ammonia-nitrogen (NH_3-N) in buffered rumen fluid, and yields of liquid-associated (LAM) and solid-associated microbial mass (SAM) (3) were determined after 24 h. All treatments were incubated in duplicate on three different days. Linear and quadratic effects of RNB were evaluated for the different carbohydrate sources using PROC GLM in SAS 9.4 with RNB as main effect. Differences between least squares means were detected by Tukey posthoc comparisons.

Results: A linear increase in GP with decreasing RNB was observed, likely due to higher proportions of degradable carbohydrates in the substrate (Table 1). The NH_3-N concentrations decreased with declining RNB for all carbohydrates. Total SCFA concentrations, LAM, and SAM were not affected by RNB, with the exception of sucrose for which SCFA increased and SAM decreased linearly with declining RNB.

Table 1 Fermentation parameters at different levels of rumen nitrogen balances (RNB, g/kg DM) tested in three carbohydrate sources during 24 h of *in vitro* incubation (least square means).

Carbohydrates	Sucrose				Corn starch				Cellulose			
Actual RNB	-0.7	-5.7	-7.8	C	-0.8	-5.9	-9.0	C	0.4	-5.1	-8.4	C
GP (ml/g DM)	102^{a}	106^{b}	107^{b}	L	109^{a}	114^{bc}	116^{c}	Q	63.9	68.2	72.0	L
NH_3-N (µg/ml)	76.4^{a}	53.7^{bc}	47.8^{b}	L	81.1	55.4	34.8	L	76.3	46.8	19.1	L
SCFA (µmol/ml)	26.7	27.9	28.2	L	27.1	27.0	27.3	-	21.6	21.7	22.9	-
LAM (mg N/2 g DM)	13.7	13.3	12.8	-	12.5	11.5	9.5	-	12.6	13.2	12.9	-
SAM (mg N/2 g DM)	1.3^{bc}	1.1^{ab}	0.9^{a}	L	1.2	1.0	1.1	-	1.5	1.4	1.5	-

DM, dry matter; GP, gas production; LAM, liquid-associated microbes; NH_3-N, ammonia-nitrogen; N, nitrogen; SCFA, short-chain fatty acid; SAM, solid-associated microbes. Values with different supercripts within a row for each carbohydrate source differ between RNB levels, whereas L and Q denote linear or quadratic contrast (C) effects of decreasing RNB levels ($P < 0.05$). - indicates a lack of effect ($P \geq 0.05$).

Conclusions: Negative RNB of ≥ -8.0 g/kg DM do not hamper *in vitro* fermentation of different carbohydrates; however, microbial protein synthesis may decrease with declining RNB due to lower nitrogen availability for microbial growth, particularly when rapidly degradable carbohydrates are fed.

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118.

Decreased phytic acid levels and viscosity in wheat bran through solid-state fermentation with *Pleurotus* fungi

Verringerung von Phytatgehalt und Viskosität in Weizenkleie durch Solid-state Fermentation mit Pleurotus Pilzen

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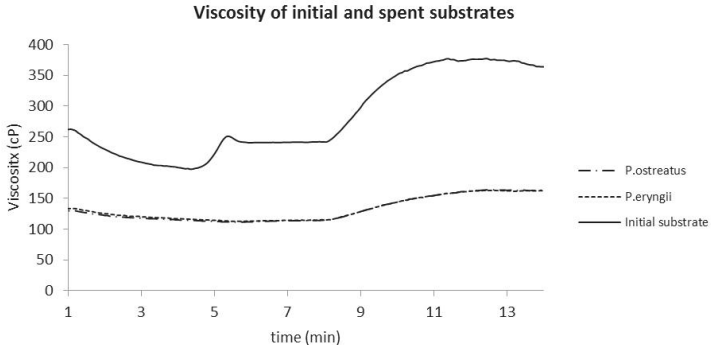
Cereals play an important role in human and animal nutrition. In parallel with growing population, the demand for cereals and cereal-based food has constantly increased. In this context, cereal technology, especially the milling process, can be regarded as one of the key technologies in the food supply chain. However, the need for sustainable use of bio-based products also has to take into account the side-streams of the Agrifood chain. Among these, wheat bran accumulates at high quantities attaining around 150 million tons per year world-wide. Due to this fact, there is some increasing need for innovative strategies to sustainably utilize and valorize wheat bran. Despite its nutritionally valuable compounds (13-18% protein, 10-14% starch, 4% lipids and 7% minerals), so far wheat bran has been mainly established as a low cost feed compound. The restricted implementation of wheat bran in animal diets results from its high amount of non-starch polysaccharides. In this respect this study was carried out to investigate the valorization of wheat bran by solid-state fermentation (SSF) using *Pleurotus* strains, having the capability to degrade lignocellulose through their lignocellolytic system. Pre-digestion of wheat bran via SSF may offer novel application fields in animal feeding, while mushrooms cultivation significantly contributes to modern nutrition.

Methods: This study was carried out at the Christian Doppler Laboratory for Innovative Bran Biorefinery, at the University of Natural Resources and Life Sciences Vienna, Austria. Fungal fermentation experiments were performed using two substrate batches containing 98% wheat bran and 2% limestone under pilot plant conditions at a moisture content of 64%. Inoculated batches were incubated in a climate room at 23°C with a relative humidity of 85% to ferment the wheat bran with two selected *Pleurotus* strains (*P. eryngii and P. ostreatus*). After 21 days, the temperature was dropped to 18°C and the relative humidity was raised to 95%. Subsequently, composition (XP, XL, XA, DM, NDF, ADF and phytic acid) of the initial and residual substrates as well as viscosity were examined. The latter parameter was monitored using a rotation viscosimeter RVA 4500 (Perten Instruments, Hamburg). Mushroom yield, biological efficiency and composition (XP and DM) were also determined. All experiments and analyses were performed in duplicates.

Results: Solid-state fermentation induced changes in XP (*P. eryngii*: +23,8%; *P. ostreatus*: +38,1%) and XA (*P. eryngii*: +57,8%; *P. ostreatus*: +28,9%), while a considerable decrease in XL was observed (-63,4%) as a result of fermentation with *P.ostreatus*. During fermentation with *P. eryngii*, the initial phytic acid concentration was reduced by -69,2% On the contrary, the substrate showed no marked change of phytic acid levels when fermented with P. ostreatus. The decrease in NDF (*P. eryngii*: -19,0%; *P. ostreatus*: -22,5%) and ADF (*P. eryngii*: +5,5%; *P. ostreatus*: +8,5%) was not pronounced, but a remarkable decrease in viscosity was observed upon fermentation with both *Pleurotus* strains (see Figure). Furthermore, mushrooms harvested from wheat bran fermented with *P.eryngii* showed increased protein and dry matter contents in comparison to those grown on commonly used substrates. *P. ostreatus* showed no fruiting body formation.



Conclusion: The results of this study indicate that solid-state fermentation with *Pleurotus* fungi offers some useful tool to modify the phytic acid content as well as viscosity. Thus, mushroom fermentation of wheat bran may enable innovative possibilities to valorize this residual substrate in animal feeding.

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119.

Expander processed maize in fattening pigs: impact of starch gelatinization on starch digestibility and microbial metabolites in ileal and colonal digestasta

Expanderbehandlung von Mais: Einfluss eines gesteigerten Stärkeaufschlussgrades auf dessen Verdaulichkeit und den Gehalt an mikrobiellen Metaboliten im Ileum und Colon von Mastschweinen

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Dietary manipulation of intestinal microbiota in pigs is possible by nutrient composition in the basal diet, feed additives and feed processing, like the high temperature short time (HTST) technology. Especially preconditioning and expander processing modify nutrient availability of feed components and changes availability of substrates for microbial fermentation in the gastrointestinal tract. Comparing expander processed materials with their raw counterparts, greater starch gelatinization values can be expected. A result of the improved starch digestion in the small intestine could be that the content of highly fermentable carbohydrates in the hindgut is reduced (1). The aim of the present study was to determine the effect of an intensive expander treatment of maize on starch digestion and microbial metabolites in the digesta of ileum and colon in fattening pigs.

Methods: For this trial 45 barrows (30.9±0.4 kg initial BW) were assigned to one of 3 experimental diets including conventional dried (C), or quantitative substitution of processed maize. The processes were short- (SC, 60 sec.), or long-term conditioned (LC, 1080 sec.) and subsequently expanded (approx. 45 kWh/t using expander Model OEK 15, Amandus-Kahl, Reinbek, Germany) maize of the same batch. Assuming no change in nutritive value due to processing, processed or unprocessed maize was mixed with further components (soybean meal, wheat bran, premix, TiO_2) at the same proportion (grower: 66%; finisher: 65%) to achieve requirements (2). Mash feed and water were provided *ad libitum* till slaughtering. Animals were slaughtered at approx. 115 kg BW. The entire intestinal tract was removed and digesta were collected from ileum and rectum to evaluate apparent ileal (AID) and apparent total tract digestibility (ATTD). Furthermore, digesta of ileum and colon were collected and immediately stored at -80°C until analysis. Short chain fatty acids (SCFA) were measured by gas chromatography (Agilent 7890A-G3440A-GC System, Santa Clara, USA). Starch gelatinization was measured enzymatically by an amyloglucosidase - kit (AMG). Discrete mean particle size (dMEAN) was determined in complete diets by dry sieving. Data were analysed using ANOVA assuming a randomized block design, with Tukey-Kramer test for LS-mean separation ($P<0.05$).

Results: The results of the starch gelatinisation of the different maize modifications, starch digestibility and microbial metabolites in ileum and colon digesta are presented in Table 1. In contrast to the AID the ATTD of starch was improved by the expander processed maize. Regarding the formation of SCFA, the amount of propionic acid increased in ileum whereas the amount of butyric acid decreased in ileum and colon ($p<0.05$). The ratio between C_2:C_3 was not altered, however an intensive conditioning treatment enhanced the ratio between C_2 and C_4 in ileum ($p<0.05$).

	C	SC	LC	SEM	p-value
starch gelatinization, %	15	70	79	-	-
dMEAN, µm	1053	870	961	-	-
AID of starch, %	94.4	95.0	94.6	0.60	n.s.
ATTD of starch, %	99.3^{b}	99.6^{a}	99.6^{a}	0.02	<0.05
Concentration of SCFA in ileum (mmol/kg DM)					
DM - ileal digesta, % DM	12.7	12.8	13.8	0.28	n.s.
Starch, % DM	5.73	5.38	4.51	0.33	n.s.
Acetic acid (C_2)	23.3	25.8	27.6	1.78	n.s.
Propionic acid (C_3)	1.0^{b}	1.2^{ab}	1.6^{a}	0.09	<0.05
Butyric acid (C_4)	2.0^{a}	1.3^{ab}	0.7^{b}	0.18	<0.05
Ratio C_2:C_3	20.9	22.5	20.1	1.68	n.s.
Ratio C_2:C_4	15.9^{b}	35.6^{ab}	59.5^{a}	4.90	<0.05
Concentration of SCFA in colon (mmol/kg DM)					
DM - colon digesta, %	23.6	24.7	24.0	0.35	n.s.
Acetic acid (C_2)	246.9	243.0	231.6	6.81	n.s.
Propionic acid (C_3)	81.0	72.4	71.8	3.17	n.s.
Butyric acid (C_4)	39.5^{a}	31.8^{b}	28.8^{b}	1.21	<0.05
Ratio C_2:C_3	3.0	3.5	3.3	0.17	n.s.
Ratio C_2:C_4	6.6	7.7	7.6	0.33	n.s.
pH-value	6.13	6.35	6.39	0.05	n.s.
^{a,b}Means within a row not sharing the same superscripts differ at $p < 0.05$					

Conclusion: There is potential to improve nutrient digestibility of expander treated maize for pigs when starch gelatinisation increases. Furthermore, the reduction of SCFA concentration in the colon suggests that several compounds are more easily digested already in the ileum, resulting in a reduced nutrient flow into the large intestine.

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120.

Effects of transport/soaking time on results of wet sieve analysis in liquid diet samples

Effekte der Transport-/Einweichdauer auf die Ergebnisse der Nassen Siebanalyse von Flüssigfutterproben

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The particle size distribution (PSD) within diets is a well-known etiologic factor for gastric ulcers in pigs. Therefore the analysis of particle size distribution is frequently requested by farmers and veterinarians. The wet sieve analysis is a common method to characterize the particle size distribution of compacted (pelleted/ crumbled) porcine diets [1]. Thus, also liquid diets were often submitted for analysis. In this context the question arises, whether the sending duration and conditions might affect the results of a standardized procedure for wet sieve analysis. In this experiment the effects of different soaking/transport durations on diets' PSD were investigated.

Material and Methods: About 40 g of a dry porcine cereal based diet (CF content:~ 3.8 % on a DM basis) in a mash as well as in a pelleted form (pellet diameter: 3 mm; identical chemical and botanical composition; equal PSD in the wet sieve analysis [1]; 48.5 % wheat, 25 % barley, 21 % soybean meal, 3.1 % mineral supplement, L-lysine, methionine, soybean oil) were soaked with 1 L distilled water for 1 h (common method of wet sieve analysis [1]), 48 h (simulating a transport duration of 2 days) and 96 h (simulation of a transport over the weekend) and sieved via a sieve tower (sieve holes 3.15, 2.00, 1.40, 1.00, 0.80, 0.56, 0.40, 0.20 mm; Company: Retsch GmbH) with 10 L distilled water. After drying at 103 °C and cooling in a desiccator sieve's weights with dried residues were measured and the PSD (in % of the DM amount used) was calculated. Statistical analyses were done using SAS software (Cary, NC, USA; PROC NPAR1WAY).

Results and Discussion: The particle size distribution of the feed samples differed markedly depending on the time of soaking. In the following table the main results are presented.

Compaction		Meal			Pellet		
Soaking, h		1	48	96	1	48	96
n		4	3	3	4	3	3
Mass of particles, % of DM	> 1 mm	49.3^{a} ± 0.60	45.5^{b} ± 1.13	39.4^{c} ± 0.67	48.5^{a} ± 1.41	42.4^{b} ± 1.81	27.0^{c} ± 1.37
	< 0.2 mm	25.3^{a} ± 3.22	31.8^{a} ± 0.73	37.3^{b} ± 1.36	26.8^{a} ± 0.78	31.7^{b} ± 0.69	50.3^{c} ± 2.83

Different superscripts (^{abc}) indicate significant differences within a row and compaction form

In particular the proportion of particles <0.20 mm increased with extended time of soaking. A soaking/transport duration of 48 h caused an increase in particles <0.20 mm of 25.7 % in the meal, and 18.3 % in the pelleted diet. After a transport of the liquid diet over a weekend (96 h soaking) the proportion of fine particles (<0.20 mm) increased by 47.4 % in the meal and by 87.7 % in the pelleted variant. Results of the wet sieve analysis after frozen storage and transport (-18 °C, data not shown) were comparable with those after 1 h of soaking. Interestingly the increase in particles <0.2 mm due to longer soaking duration was markedly lower in the mash diet compared to the pelleted one.

Conclusion: In conclusion, submitting samples of diet in a dry form is recommended or when there is no possibility to send the dry original material, storage at -18 °C and frozen transport should be chosen. Based on the experimental findings it is worthwhile to get information about the "history of a liquid sample", for example a 24 h lasting "controlled fermentation" as more and more common could influence the diet's physical form at ingestion posing a risk for gastric ulcer. There is also a forensic aspect that earns to be considered: The diminution due to longer soaking and mixing is not in the area of responsibility of the feed producers but to the person offering the diet to the animals.

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121.

Effect of replacement of soybean meal by rape seed meal plus peas in feed on laying performance of two laying hen strains

Einfluss eines Austausches von Sojaextraktionsschrot gegen Rapsextraktionsschrot und Erbsen im Futter auf die Leistungsentwicklung von zwei Hennenlinien

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Soybean meal is the most common protein source in diets of poultry species. The majority of this soybean meal is imported from non-European countries and often derived from genetically modified varieties. Therefore, in Europe, the interest has increased to research on the use of alternative local protein sources derived from non-genetically modified varieties, such as rape seed and grain legumes. In German, in 2014, rapeseed was grown on 1.39 million ha contrasting with 41.7 thousands ha used for feed peas. Legumes like peas could be used as an alternative high protein source in poultry diets. Objectives of this study were to test the effect of a total replacement of soybean meal with rape seed meal plus peas in hens' feed on laying performance and egg quality parameters of two strains of hens with different laying performance.

Methods: 230 Lohmann Brown (LB) and 230 Lohmann Dual (LD) hens were randomly divided into 4 groups. The hens were kept in pens (23 hens per pen) with 5 pens per group. The study commenced when the hens were 22 weeks old and continued until the 6th laying month (168 days). Each hen was offered the respective diet and water ad libitum. Number of eggs laid per pen was recorded daily and the feed consumption monthly. Each month the collected eggs were weighed four times within two weeks. In the 2th, 4th and 6thlaying month, all eggs laid per pen over 3 consecutive days were collected to measure egg composition. In the treatment, soybean meal feed (SBM - 21.6%) was totally replaced by 12% rapeseed meal (REM - 8.2 mmol glucosinolate/kg) plus 35% pea "James" (235 g crude protein/kg). All diets contained a balanced concentration of crude protein, essential amino acids Lys, Met, Thr, Trp and metabolisable energy. Data were analyzed via ANOVA (SAS) and the Student-Newman-Keuls-test (p

Results and conclusion: Daily feed intake, laying intensity and egg weight of LB hens was significantly higher compared with LD hens. While no effect of substituting SBM through REM/peas in the diet was seen on laying intensity, the egg weight of LB and LD hens was decreased. The daily egg mass production was lower in the LB REM/peas group compared with LB SBM hens and not significantly different between the two LD groups. During the complete trial period eggs of LD hens showed a significantly higher percentage of egg yolk and as a result a reduced part of egg white.

Table 4: Laying performance of hens - feed intake (FI), laying intensity (LI), egg weight (EW), egg mass (EM), feed conversion (FC) (LS Means)

Table 1: Content of short chain fatty acids in ileum and colon digesta (mmol/kg dry matter)

Treatment	Hen	FI (g/d)	LI (%)	EW(g/egg)	EM(g/hen/d)	FC (kg/kg)
1 SBM	LB	127.6 a	92.0a	63.2 a	58.2 a	2.196 c
2 REM/peas	LB	125.0 a	92.6 a	61.3 b	56.8 b	2.205 c
3 SBM	LD	110.2 c	82.1 b	58.4 c	47.7 c	2.309 b
4 REM/peas	LD	115.6 b	83.8 b	57.0 c	47.5 c	2.433 a
ANOVA, P value						
Diet		0.3	0.3	0.002	0.1	0.009
Hen		<0.001	<0.001	<0.001	<0.001	<0.001
Diet x Hen		0.003	0.6	0.6	0.2	0.02

a; b; c; - Means with different letters differ significantly

Conclusion: The trial's results allow the conclusion that a total replacement of soybean meal with rape seed meal plus peas in feed of LB hens first of all reduces the egg weight and secondly declined the feed conversion of LD hens.

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122.

Influence of an increasing content of blue sweet lupines (*Lupinus angustifolius*) in the feed on the growth of broiler chickens

Einfluss einer steigenden Konzentration an Blauen Süßlupinen im Futter auf das Wachstum von Mastbroilern

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Soybean meal is the most common protein source in diets of broiler chickens. The majority of this soybean meal is imported from non-European countries and often derived from genetically modified varieties. Therefore, in Europe, the interest has increased to research on the use of alternative local protein sources derived from non-genetically modified varieties, such as grain legumes. The international meaning of the legumes is underlined by the 68th UN General Assembly declaring 2016 the International Year of Pulses. Blue sweet lupines are suited as ingredient of poultry feed due to their high protein content and low alkaloids level. Processing treatments such as heat processing do you carry out with the aim to improve the digestibility of nutrients. Objectives of this study were to test the effect of a replacement of the protein source soybean meal (SBM) with legumes as lupines as seeds or after toasting in broilers' feed on performance.

Methods: One-day old male 400 cockerels (ROSS) were randomly allocated into 5 treatment groups (8 pens/group) over a study period of 35 days. Feed and water were provided for ad libitum consumption. Live weight was recorded for each broiler individually whereas feed was weighed back weekly on a pen-basis. Soybean meal (32%) was gradually replaced by lupines (15/30%) as seeds or after toasting in the diets. The low-alkaloid lupine variety "Borlu" contained 297/317 g/kg crude protein (seed/after toasting). All diets contained a balanced concentration of 21 % crude protein and essential amino acids (Lys, Met, Thr, Trp). N-balance trial was carry out with 9 replicates per group 1, 3, 5 with highest lupine concentration (Table 1), with broilers from the same hatch at the age of 3 weeks and calculated the Productive Protein Utility (PPU, %)=(N-balance / N-intake)*100. Data were analyzed via ANOVA (SAS) ($p<0.05$).

Results and conclusion: The results of this study indicate that an inclusion rate up to a level of 15% blue sweet lupines or 15% toasted blue sweet lupines in broilers' diets was without negative effects on feed intake, growth performance, percentage of carcass (n=8 per group) and protein utility (n=9 per group) (Table 1).

Table 1 Feed intake, final body weight, feed to gain ratio, carcass and productive protein utility (PPU) of broilers (LS means)

Group	Diet proportion (%)			Feed intake	Body weight	Feed to gain	Carcass	PPU
	Soya	Lupine,crude	Lupine,toasted	(g/d)	(g)	ratio (g/g)	(%)	(%)
1	32	-	-	80.2	2163	1.325	69.4	73.2
2	28	5	-	84.2	2108	1.427	67.9	-
3	20	15	-	87.2	2164	1.438	68.0	72.6
4	28	-	5	84.4	2127	1.416	67.9	-
5	20	-	15	85.2	2100	1.448	67.6	73.6
Anova, P value								
Lupin, Crude/toasted				0.6	0.3	0.9	0.8	0.4
Lupin (%), 0/5/15				0.4	0.5	0.5	0.9	-
Lupin x Concentration				0.6	0.08	0.8	0.6	-

Conclusion: The trial's results allow the conclusion that a replacement of soybean meal with blue sweet lupines up to a level of 15 % does not affect the growing performance of broilers and toasting of lupines seed does not result in a better chicken performance.

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123.

Effect of varying dietary protein-levels on performance and total body composition in dual-purpose chickens

Einfluss verschiedener Proteingehalte im Futter auf die Leistung und Ganzkörperzusammensetzung von Zweinutzungshühnern

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Question: Dual-purpose chickens were introduced to the market as an alternative to avoid culling of day-old male chicks of layer breeds to improve animal welfare. However, there are few data regarding nutrition of dual-purpose chickens. The aim of this study was to investigate whether different levels of dietary protein have an effect on performance and total body composition of male dual-purpose chicken.

Material and methods: In total, 480 male Lohmann Dual day-old chicks were fed three different diets over a period of 77 days. Broilers were allocated to 24 pens (20 birds per pen) and fed three different diets resulting in 8 replicates per diet. Diets were formulated to be isoenergetic and contained three different protein levels: The control diet (C) contained 23 % crude protein in the starter diet, 21.6 % in the grower diet and 19.4 % in the finisher diet, according to recommendations for conventional broilers (1). The remaining two diets were reduced in protein by 5 (LP1) or 10 percent (LP2), having 22.1 % or 20.4 % of crude protein in the starter diet, 20.3 % or 19.2 % in the grower diet and 18.6 % or 17.5 % in the finisher diet. During the whole experiment the animal performance (body weight, body weight gain, feed intake, and feed conversion ratio) was recorded weekly on a pen and individual basis.

Analysis of diets, as well as full body analysis, was performed according to standard methods (2).

Statistical analysis of the results was conducted using SPSS. Shapiro-Wilk test was used confirming normal distribution, one-way ANOVA and a post-hoc Tukey-test were used to test differences between the three feeding regimes.

Results: Protein reduction had no effect on performance data (Body weight: $p = 0.066$; FCR $p = 0.406$). Animals of the control group had a final body weight of 2221 g (± 544 g) and a FCR of 3.19 (± 0.32). Highest body weights and lowest FCR were reached by animals of LP1 (BW = 2270 g (± 544 g), FCR = 2.96 (± 0.26)), animals of LP2 reached final weights of 2091 g (± 595 g) and a FCR of 3.07 (± 0.42). Analysing the individual weights of the animals showed a split in the population development. As there was a clear difference in the data between high and low performing birds, each group diversified into two groups (Fig. 1). Body composition analysis showed no difference in crude ash, crude protein, crude fat, calcium and phosphorus.

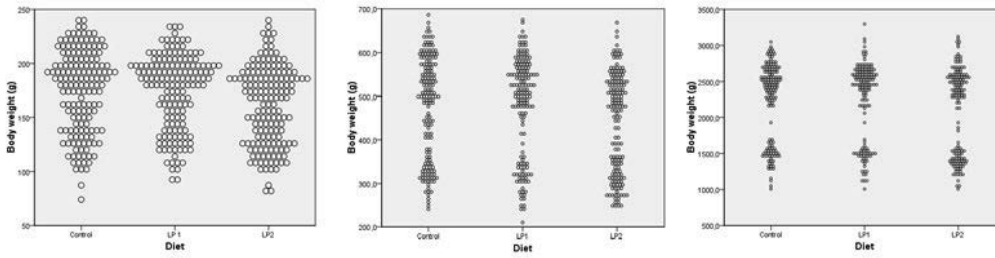

Figure 1. Development of individual body weights in 14, 28 and 77 day old Lohmann Dual chickens

Conclusion: The results of this study demonstrated that a reduction of dietary protein by 5 and 10% had no negative impact on animal performance and total body composition in dual-purpose chickens. During the fattening period the animals showed a large deviation regarding body weight, which might be due to genetic inhomogeneity.

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124.

Impact of variously processed feeds on gut-associated and peripheral immune cells of broilers

Einfluss unterschiedlich technisch behandelter Futtermittel auf das Darm-assoziierte und periphere Immunsystem von Broilern

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Differently processed feeds were shown to influence intestinal morphology and nutritional physiology while effects on gut- associated immune cells as components of the intestinal barrier were only rarely examined. Studies of Liu et al. (1) with broilers give evidence that particle size can affect immune cells of the intestine. Therefore, the present study focused on the effects of variously processed feeds on the immune system of broilers. Special attention was attached on the local immune system of the intestine and the caecal tonsils (CT).

Methods: A total of 36 male broilers (308 Ross) were fed a basal feed (maize/soybean meal/wheat) differing in particle size (Cg - coarse, d50 (cumulative particle size distribution at 50 %) = 1.65 mm vs. Fg - fine, d50 = 1.04 mm) and in hydro-thermal treatment (HTT) (M - meal, without; P - pelleted; ExP - expanded and re-pelleted) for 28 days. Initially, broilers were housed in groups in floor pens on chopped straw. Starting at the 14th day of life they were housed individually in metabolic-single cages without bedding. Feed and water were available *ad libitum*. Animals were slaughtered at the 28th day of life. Blood samples were collected and CT and jejunal tissues were dissected for isolation of leucocytes. Differentiation of peripheral blood leucocytes was based on counting of blood smears. Isolation of cells from lamina propria of jejunal tissue was performed according to modified methods of Davis et al. (2). Cells of CT were prepared by cutting the tissue and collecting of cells in PBS. Cell suspension was separated from remaining tissue parts by using washing steps and cell sieves. T-cell phenotyping was carried out by staining peripheral and intestinal T-lymphocytes with monoclonal antibodies for CD3, CD4 and CD8 and flow cytometric measurements. For statistical analysis MIXED procedure of SAS Enterprise Guide 4.3 was used.

Results: Total counts of blood leucocytes were significantly higher in animals fed Cg feed compared to broilers fed Fg feed ($p = 0.034$). Proportions of peripheral lymphocytes were significantly higher in animals fed Fg feed compared to animals fed Cg feed ($p = 0.019$). Furthermore, they were significantly higher in animals fed P feed compared to animals fed ExP feed ($p = 0.030$). An opposite effect was observed for the proportions of heterophile granulocytes. While total counts of blood lymphocytes were not affected by treatment heterophile granulocyte counts were significantly higher in animals fed Cg feed compared to animals fed Fg feed ($p = 0.018$). T-cell subsets of blood or lamina propria were not different between feeding groups ($p < 0.05$). However, a significant effect of HTT on the $CD3^+/CD4^-/CD8^-$ T cells of CT was observed (Table). In particular, this population tended to be higher in animals fed meal compared to animals fed P feed or ExP feed ($p < 0.1$).

Particle Size	HTT	T-cell subsets of Ceacal tonsils [%]*			
		$CD4^+/CD8^-$	$CD4^-/CD8^+$	$CD4^+/CD8^+$	$CD4^-/CD8^-$
Coarsely ground	M	15.3	43.9	10.4	30.4
	P	16.8	50.3	9.7	23.3
	ExP	22.7	42.3	9.3	25.8
Finely ground	M	19.0	37.0	6.3	37.7
	P	17.0	49.7	8.0	25.3
	ExP	15.2	51.5	12.2	21.1
		p-Value			
Particle size		0.569	0.868	0.667	0.666
HTT		0.680	0.104	0.653	0.043
Particle size x HTT		0.095	0.190	0.444	0.411

*LSMeans; n = 6

Conclusion: The present results indicate that feeding differently processed feeds can influence white blood cell counts and modulate local immune cells of broilers. Consequences of these findings need to be examined further.

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The effect of dietary cereal type, crude protein content and butyrate application on selected markers of metabolism in broiler chickens

Die Wirkung des Getreidetyps, des Rohproteingehaltes und der Butyratsupplementierung auf ausgewählte metabolische Parameter bei Broilern

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Since the banning of use of antibiotics as growth promoters in the European Union in 2006, alternative feed additives, such as the short chain fatty acid butyric acid (butyrate) are successfully applied in poultry nutrition. Butyrate exerts its numerous beneficial effects either mixed in the feed of animals (exogenous origin) or produced by anaerobe microbial fermentation in the caeca of birds (endogenous or caecal origin). The latter can be enhanced by feeding diet rich in non-starch polysaccharides (NSP), especially when supplemented with NSP-degrading enzymes, ameliorating disadvantageous effects of NSP and providing more substrates for butyrate production by enzymatic cleavage of long chain carbohydrates. Further, reduction of dietary crude protein (CP) content is also an important issue in broiler nutrition. In the present study we aimed to investigate the age-related metabolic responsiveness of broilers to maize- or wheat-based diets (highly different in their soluble NSP content), as well as to the dietary CP content and butyrate supplementation.

Methods: Ross 308 male broiler chickens were assigned to eight dietary groups, fed with maize-based (MB) or wheat-based (WB) diet supplemented with xylanase and glucanase enzymes, representing low and high NSP levels; with dietary CP content meeting standard requirements of appropriate dietary phase ("normal protein" [NP] groups) or reduced by 15% ("low protein" [LP] groups, diet completed with free limiting amino acids to avoid growth depression caused by inadequate amino acid supply). Further, diets were formulated with or without sodium (n-)butyrate supplementation in the dose commonly applied in poultry nutrition (1.5 g/kg diet). Blood samples were taken from the brachial vein at the age of 7, 21 and 42 days (week 1, 3 and 6 samplings, n=10/group per time point). Plasma concentrations of total protein (TP), albumin, uric acid, as well as activity of aspartate aminotransferase (AST) and creatine kinase (CK) enzymes were measured spectrophotometrically by an automated apparatus, concentrations of glucose and triglyceride (TG) were determined by colorimetric methods, further, those of glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and insulin were assessed by chicken-specific sandwich ELISA tests. Analysis of data was performed by multi-way ANOVA and pairwise comparison using R 3.2.2 software.

Results: Total protein concentration was significantly higher in groups fed with WB diet ($p<0.001$) or containing NP ($p<0.001$) on week 3; albumin/TP ratio was reduced by butyrate supplementation ($p=0.022$) on week 1. Plasma uric acid level was increased by WB diet ($p=0.022$) on week 1; by WB diet ($p<0.001$), NP ($p<0.001$) and butyrate ($p=0.048$) on week 3 and by NP ($p=0.002$) on week 6. AST activity was stimulated by WB diet ($p=0.042$) on week 3, CK activity was reduced by NP on week 3 ($p=0.004$) and 6 ($p=0.041$). Blood glucose level decreased in groups receiving WB diet ($p=0.002$) on week 3, and parallelly, TG concentration augmented ($p=0.011$) in the same groups on week 3. In case of GLP-1, GIP and insulin, no response to any of the investigated nutritional factors could be detected. On week 6 compared to week 1 measurements, we also found that uric acid concentration decreased by 50% ($p<0.001$), CK activity was elevated thirteen-times ($p<0.001$), and concentrations of GLP-1 and insulin increased by 60% ($p=0.007$) and by 30% ($p<0.001$), irrespective of dietary treatment.

Conclusion: Nutritional factors could generally alter values of interest at the age of 3 weeks, indicating a metabolism more sensitive to diet-associated regulatory mechanisms compared to week 1 or 6, possibly due to the intensive growth of the animals. We also found type of basal diet, highly different in soluble NSP content effective to influence several biochemical parameters, presumably through altered caecal microbial SCFA, primarily butyrate production. Further, no adverse effect of dietary CP reduction on growth or health of animals was detected.

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Comparison of standard feedstuffs for carps and koi fish with regard to nutrient composition and requirements

Vergleich handelsüblicher Karpfen- und Koi-Futtermittel hinsichtlich Nährstoffzusammensetzung und Bedarfsdeckung

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In recent years the keeping of koi fish has gained popularity in Germany. Although predominantly privately owned, koi fish might be fed requirement-based with standard feedstuffs for carps in aquaculture, as they belong to the family of the cyprinids. Therefore, the aim of the study was to compare carp and koi fish feedstuffs regarding the composition of nutrients and hence the fulfilment of nutrient requirements.

Methods: Commercial feedstuffs for semi-intensive and intensive rearing of carps (n=10) and koi fish feed (no winter feed, n=10) were randomly selected. Proximate analyses followed methods of VDLUFA (1) and amino acids (AA) were determined in acid or oxidized hydrolysates by HPLC. The content of digestible energy (DE) was calculated according to (2). A one-way Analysis of Variance was carried out for all analysed variables as well as calculated values for $P<0.05$ (SPSS, version 23.0). Contents of crude protein (CP), crude fat (CL), starch and soluble P as well as the sum of essential AA and the DE/CP ratio were taken into account to carry out a ranking of all 20 feedstuffs, whereas starch and P were evaluated positively with low and the other variables with high contents.

Results: The crude nutrient contents (CP, CL, crude fibre [CF], ash and nitrogen free extracts) of the selected feedstuffs for carp and koi fish vary widely within the same group, indicating a representative selection of each type. However, the analysis of variance revealed no differences ($P>0.05$) between carp and koi fish feedstuffs within the same crude nutrient. Concerning the declared values of CP, CL, CF and ash, in 10 feedstuffs (4 for carp, 6 for koi fish) deviations above or below the declared contents for one or more nutrient within the same feedstuff were analysed according to the directive 767/2009/EG for allowed tolerances in mixed feed.

In both feed types the total content of essential AA exceeded the requirement (3) of 120 g/kg dry matter (DM) with 172 g/kg DM in carp feed and 157 g/kg DM in koi fish feed. However, regarding single AA, in 4 feedstuffs of carp feed the contents of lysine, methionine and threonine did not meet the animals' demands, whereas in koi fish feed the contents of lysine, phenylalanine and valine did not fulfil the requirements in any of the tested feedstuffs. In accordance to these findings, differences ($P<0.05$) for lysine, methionine, phenylalanine, threonine, valine and as well tyrosine and histidine were found between feedstuff types.

The recommended DE/CP ratio of 0.4-0.5 MJ DE/g CP was reached in 6 carp and 5 koi fish feed samples, but generally DE/CP ratios did not differ ($P>0.05$) between feed types. For the six most important quality parameters (CP, sum of essential AA, DE/CP, CL, starch and soluble P) an overall evaluation was carried out. According to this assessment a feed for intensive aquaculture carp rearing, a feed for intensive rearing of koi fish and another feed for intensive carp rearing were ranked in first, second and third place, respectively, whereas 2 other koi fish feedstuffs were among the first 10 places in the ranking.

Conclusions: Regarding the nutrient demands for cyprinids, the carp feedstuffs show a more favourable composition with on average higher CL contents and a better AA profile as well as DE/CP ratio. However, the energy content in protein-rich carp and koi fish feed is considered to be too low, which might lead to excessive nitrogen excretions. In general, differences between analysed parameters were low, so that from the nutritional point of view commercial carp feedstuffs for aquaculture might be used for koi fish in accordance with the requirement-based nutrient composition.

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127.

Insect protein in aquafeed - effect of substituting soy protein on protein quality of Tilapia feed

Insektenprotein im Fischfutter - Einfluss einer Substitution von Sojaprotein auf die Proteinqualität von Tilapiafutter

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Fishmeal (FM) and soybean protein are the most common protein sources used in fish feed, but associated with environmental, economic or technological problems (1). Due to the rapid development of aquaculture the increasing demand for feed has initiated research about alternative and more sustainable proteins for aquafeeds. Recent investigations suggest that insect meals could be an interesting option. The aim of the current study was to evaluate protein quality parameters following substitution of soybean protein by partly defatted insect meal from larvae of black soldier fly (*Hermetia illucens*) in Tilapia diets.

Methods: A growth study was conducted with 400 juvenile all-male fishes of Nile tilapia (*O. niloticus*) using a control diet (8% FM, 37% soy protein concentrate (SPC; Crude protein (CP): 74% of dry matter (DM)), and four experimental diets with 25, 50 or 100% replacement of SPC by partly defatted (Crude lipid: 14% of DM) *Hermetia* meal (CP: 61% of DM). Diets (HM25, HM50, HM100) were formulated to be similar both in CP (43% of DM) and energy content. Essential amino acid supply of the diets was within the recommendations for Tilapia. Nevertheless, diet HM100 was formulated in duplicate, without or with supplemented Lys to check if any Lys limitation could be excluded. Growth response and protein utilization were studied in a semi-closed in-door water recirculation system with 20 tanks (320 l/tank; water temperature 25.0 ± 0.2°C; regulated photoperiod 14h light/10h dark). Four replicate tanks per diet (20 fish per tank) were utilized in a 56 d growth experiment by DM provision in two meals each at 2.2% body mass (BW). Ten fish at the beginning and 12 fish per diet at the end of the growth study were analyzed for body composition to generate N deposition data. Both parameters of growth response and protein quality were calculated according to (2, 3). Standardized net protein utilization (PNu_{std}) referred to an average of daily N intake ($NI=560mg/BWkg^{0.67}$) as observed. Statistical analyses (one-way ANOVA, Tukey-test) were conducted by R-software (version 3.0.2).

Results: All diets were very well accepted. Replacement of SPC by HM slightly improved protein quality (PNu_{std}) as well as specific growth rate (SGR) and feed conversion ratio, but significantly only for SGR (Table). SPC replacement level of 25-50% was most effective for all parameters. Supplementation of Lys at HM100 did not result in superior protein quality, indicating that 37% HM in tilapia diets caused no Lys limitation.

Diet	Control	HM25	HM50	HM100	HM100+Lys
PNu_{std} [%]	31.8 ± 1.9	34.6 ± 1.2	34.6 ± 1.3	33.9 ± 1.8	32.6 ± 1.4
SGR [%]	3.38^{ab} ± 0.11	3.51^{b} ± 0.09	3.46^{ab} ± 0.09	3.24^{a} ± 0.21	3.31^{ab} ± 0.06
FCR [g/g]	1.98 ± 0.18	1.84 ± 0.10	1.83 ± 0.11	2.08 ± 0.20	1.99 ± 0.11

Means (± SD); SGR = specific growth rate; FCR = feed conversion ratio; PNu_{std} = standardized net protein utilization (standardized daily N-intake = $560mg/BW_{kg}^{0.67}$); different superscript letters reveal significant differences between diets ($p<0.05$)

Conclusion: Replacement of SPC by partly defatted *Hermetia*-meal up to a level of 50% tended to improve dietary protein quality of Tilapia diets under study and enhanced growth performance of Nile tilapia. Therefore, insect protein from *Hermetia illucens* can be a further option to make aquafeed formulation more flexible and sustainable when approved as feed ingredient. Ongoing research will examine the conditions for further improvement of the dietary amino acid balance in fish diets with alternative protein sources.

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128.

Struvite urolithiasis in a dog - A case of pet owner's compliance

Struvitsteine bei einem Hund - Ein Fallbericht zur Einhaltung einer Fütterungsempfehlung

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One of the most frequently seen urinary calculi in dogs are struvite stones, especially young female dogs are affected. Epidemiologically these uroliths are associated with a bacterial infection of the urinary tract with urease producing bacteria; in some cases a sterile development is discussed. Dietary levels of minerals (P, Mg) and nitrogen may influence the urine composition; protein metabolism as well as acidifying or alkalizing substances can affect urinary pH values and thus have an impact on the development of concrements: In neutral or basic urine, complexes of calcium, magnesium, ammonia and phosphate are formed because the solubility is low. Dogs suffering from urolithiasis show pollakiuria and haematuria in some cases. When struvite stones are proved in urine, a medication with antibiotics is indicated when bacteria are detected. Further on, the water intake should be increased to lower the specific gravity of urine and thus increase solubility. A dietary treatment can help to dissolve urinary stones and to avoid recurrences.

Table 1: Composition of the diet

Feedstuff	1st diet (Dec 15) amount (g/day)	2nd diet (Sept 16) amount (g/day)
beef	45	45
potato (cooked)	80	100
rice (cooked)	20	
vegetables	15	15-20
oil	5	5
mineral feed[1)]	1	1
calcium citrate	0.5	0.5
rumen, cleaned		10-15

[1)]23.6% Ca, 6.5% P, 1.0% Na, 1.4% Mg, 1.6% Cl, 0.9% S

Methods: A 20 months old female dachshund, 4 kg body weight, was presented with struvite urolithiasis. A bacterial urinary infection was successfully treated. The urinary pH value was about 8, earlier measurements showed pH values around 6.5 to 7. A commercial diet was offered but after the dog refused to consume it, the owner wished to feed a homemade diet. A diet was calculated according to the recommendations of [1] and [2] with <7g protein, <200 mg phosphorus, and <40 mg magnesium per MJ ME [3].

Results: After the first diet was fed, urinary pH value was lower (around pH 6.5) and no urine stones were detected. After some weeks the dog's owner did not see the necessity anymore to separate the dog during eating from the second dog of the family (which got a "pancreatitis diet") and so it did not only receive the specific diet. Further on, no snacks were included into the calculation, but given by the owner in larger extends. In September the dog suffered from struvite urolithiasis again, once more urinary pH value reached 8 and specific weight of the urine was >1,032 mg/ml. Thus, a second diet was calculated by replacing the amount of rice by potato and including cleaned rumen for treat.

Conclusion: After feeding the first diet for about 10 weeks, a change in urine pH was reached and no uroliths were present. The importance of treats for dog owners was not met satisfactory in the first calculation. To be successful in dietary treatment in pets, it is necessary to get to know the owner's premises and feeding practises to inform him on the one hand on how to deal with the dietary treatment and on the other hand to try to include these practises into the calculation of the diet to optimize the owner following the recommendations.

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Effect of *Hermetia illucens larvae meal as a protein source on the nutrient digestibility in dogs*

Einfluss von Hermetia illucens Larvenmehl als Proteinquelle auf die Nährstoffverdaulichkeit bei Hunden

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In recent years the occurrence of allergy or hypersensitivity against feed antigens in dogs was reported with increased frequency. Therefore, it is important to find new protein sources and to examine their usability. Larvae of *Hermetia illucens* (Black Soldier Fly) were studied as protein source for pigs, poultry or fish. Also, *Hermetia illucens* larvae are already a component of some commercial dry food for dogs, but to our knowledge, no studies regarding the apparent digestibility and the influence on fecal parameters exist yet. The hypothesis of this study was that the apparent nutrient digestibility of a diet containing *Hermetia illucens* larvae will be comparable with a commercial test diet containing lamb meal as main protein source.

Methods: Twelve beagle dogs were included in the cross-over study and divided into two groups (3 male and 3 female each). The dogs received either a diet with the animal protein source *Hermetia illucens* larvae (experimental diet) or a diet with lamb meal (control diet) as animal protein source. Main components of the experimental diet were rice meal, *Hermetia illucens* larvae meal, barley, and potato protein. In the control diet rice meal, lamb meal, barley and rice protein were the major components. Table 1 gives an overview on crude nutrients.

Table 1: Chemical composition of the diets (provided by Vet-Concept, Föhren, Germany)

	Experimental diet	Control diet
	g/ kg in dry matter	
Crude protein	260	269
Crude fat	74.9	90.8
Crude ash	54.8	84.6
Crude fibre	23.6	14.3

Both diets were fed for five weeks. Feces samples were collected at the end of each feeding period and the apparent nutrient digestibility, the fecal consistency and the daily feces amount were determined. The fecal scoring based on a 5-point scale (1: hard dry pellets - 5: watery, liquid; Middelbos et al., 2007). The determination of the crude nutrients was performed according to standard Weende procedures. Titanium dioxide was used as an indigestible marker. Statistical analysis was performed with SPSS Statistics 21 software. Data were analysed with the general linear model (univariate). Feeding group and feeding passage were considered. The level of significance was set at $p < 0.05$.

Results: The apparent digestibility was higher ($P < 0.001$) in the experimental diet containing larvae meal compared to the control diet for dry matter (93.8 and 92.9%, respectively). The apparent digestibility of other nutrients did not differ between the experimental and control diet (e. g. crude protein 77.3% and 79.2%; crude fat 97.1% and 97.3%). The daily amount of feces did not differ between the diets. The dogs receiving the experimental diet had a higher ($P = 0.044$) fecal score (2.25) compared to the dogs which were fed the control diet (2.04). However, both groups had a formed and dry stool.

Conclusion: The results of the apparent nutrient digestibility and some fecal parameters did not show clear differences between the experimental diet with *Hermetia illucens* and a commercial dry food for dogs. This could indicate that *Hermetia illucens* might be as a possible alternative protein source for dogs. However, as we can´t completely exclude an influence of the different chemical composition (e.g. crude fibre) on the nutrient digestibility, further trials, especially long-term trials should be performed to confirm these results.

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130.

Do maternal dietary omega-3 or omega-6 fatty acids affect embryo-maternal communication and preimplantation embryo development in Angus heifers?

Beeinflusst die maternale Aufnahme von omega-3 oder omega-6 Fettsäuren die embryo-maternale Kommunikation und die Embryonalentwicklung während der Präimplantationsphase bei Angusfärsen?

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As direct contact of mother and embryo is established only from day 18 on in bovine, previous embryo-maternal communication is crucial for preimplantation embryo development and establishment of pregnancy. Communication is dependent on factors in the uterine histotroph, which also provides nutrients for the embryo before implantation. Dietary omega-3 (ω3) and omega-6 (ω6) fatty acids (FA) are precursors for prostaglandins (PG), that play an important role as signaling molecules in embryo-maternal communication. By interaction with transcription factors such as peroxisome proliferator activated receptors (PPARs), ω3 and ω6 FA may also affect gene expression in the endometrium as well as the embryo, thereby modulating the composition of the histotroph. We investigated whether rumen-protected ω3 and ω6 FA accumulate in endometrial tissue of growing Angus heifers, change the composition of the histotroph and impact on embryo-maternal communication and embryo development.

Methods: Two groups of Angus heifers were supplemented with either 450 g of ω3 FA (ω3 group; n= 22) or ω6 FA (ω6 group; n = 15) per day, respectively. Following synchronization and artificial insemination, animals were slaughtered at day 15 of gestation. The embryo, uterine fluid, endometrium, corpus luteum and plasma were sampled. Plasma and endometrial FA content was determined using GC-MS. Uterine metabolome including PG and amino acids was determined using LC-MS/MS. Plasma progesterone and LDL cholesterol levels were measured using ELISA and a colorimetric assay, respectively. Gene expression changes in endometrium and corpus luteum were investigated via qPCR. The least-square ANOVA GLM procedure in SPSS (version 22 was used to analyze the effects of the supplement as a fixed factor, embryo length as a random factor, and supplement by embryo length interaction. For comparisons between diet groups that were not related to embryo length, data following a Gaussian distribution were analyzed by Student's t test. In case of a non-Gaussian distribution, a Mann-Whitney U test was performed

Results: Endometrial arachidonic acid (AA, ω6), the precursor for PG, was significantly reduced ($p$pp=0.017). Despite the reduced availability of endometrial AA in the ω3 group, neither $PGF_{2\alpha}$ nor PGE_2 differed between diet groups when comparing embryos of comparable length. However, uterine $PGF_{2\alpha}$ and PGE_2 increased in both groups with increasing embryo length. Uterine amino acids and gene expression of specific amino acid transporters (SLC1A5, SLC7A1) correlated significantly with increasing embryo length in the ω3 but not in the ω6 group. Endometrial expression of genes involved in PG synthesis (PLA2, COX2), PPAR signaling (PPARα, PPARβ, PPARγ), insulin signaling (IGF1, IGF2, IGF-1R, IGF-2R) and antioxidant defense (Catalase, SOD1 and 2, NQO1, GPx1 and 4, GSTa2) was not differentially regulated between diet groups. Plasma progesterone (P4) profiles indicated higher P4 concentrations on day 6 of pregnancy of animals where longer embryos (>3 cm (tubular and filamentous)) were recovered at day 15 of pregnancy. In addition, animals with very long embryos (>10 cm (filamentous)) showed higher P4 concentrations on day 15 of pregnancy compared to animals with shorter embryos (Conclusion: Modulating embryo elongation via maternal nutrition may impact on embryo-maternal communication during the preimplantation period and possibly further establishment of pregnancy. Until now, it cannot be stated why embryos were differentially elongated in the two diet groups. Possible underlying mechanisms such as effects of ω3 and ω6 FA on further uterine signaling molecules (e.g. cytokines) and on embryonic gene expression are currently under investigation.

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Acylcarnitine profiles in serum and skeletal muscle and mRNA expression of the carnitine acyltransferases - CPT1B and CPT2 in muscle of dairy cows around parturition

Peripartale Acylcarnitinprofile im Serum und Skelettmuskel sowie die mRNA-Expression der Carnitin-Acyltransferasen - CPT1B und CPT2 im Muskel von Milchkühen.

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The transition period from late gestation through early lactation is characterized by the mobilization of body reserves, in particular fat from adipose tissue, leading to a marked increase in circulating concentrations of nonesterified fatty acids (NEFA). Skeletal muscle with high levels of fatty acid (FA) uptake turns into the main site of oxidation of fat-derived fuels. Energy production from FA requires the transport of FA into the muscle mitochondria through a carnitine-dependent transport shuttle regulated by carnitine acyltransferases, i.e. carnitine palmitoyltransferase 1 (CPT1; present in the mitochondrial outer membrane), and CPT-2 [(located on the matrix side of the inner membrane); (1)]. Deficiencies in these enzymes or impaired functions may lead to incomplete mitochondrial FA oxidation, resulting in accumulation of acylcarnitines (AC), which may be associated with development of insulin resistance (1, 2). Our objective was to investigate the changes in the expression of muscle carnitine acyltransferases in conjunct with free carnitine and AC profiles in serum and muscle of dairy cows during the transition from late gestation through early lactation.

Methods: Twenty-one German Holstein cows were assigned to either the treatment group (n=11) or control group (n=10) and fed 100 g/d of the conjugated linoleic acid (CLA; Lutrell pure, BASF, Germany) or control fat supplement (Silafat, BASF), from days in milk 1 to 182, respectively. Biopsies from semitendinosus muscle and blood were collected on day (d) -21, 1, 21, and 70 relative to calving. The AC profiles in muscle and serum were determined by FIA-ESI-MS/MS profiling through targeted metabolomics using the Biocrates Absolute IDQ p180 Kit. The serum concentrations of insulin, NEFA, and glucose were measured and an index estimating insulin sensitivity (RQUICKI) was calculated. The mRNA abundance of carnitine palmitoyltransferase 1B (CPT1B; muscle isoform) and CPT2 were quantified by qPCR. Preliminary statistical evaluation did not show any significant effect of CLA supplement on the tested variables. Thus, CLA supplementation was disregarded as effect in the model for the final statistical analysis of the data. Data were analyzed by the MIXED procedure of SAS with time as fixed effect and cow as random effect. The threshold of significance was set at $P < 0.05$.

Results: The serum concentration of carnitine decreased with the onset of lactation. Serum acetylcarnitine (C2), and odd-chain species including C3, C3-DC, C5-OH that are produced during amino acid catabolism, were elevated around parturition compared to d 70. The serum long chain AC including C16:0, C18:0 and C18:1 concentrations were significantly enhanced ($P < 0.05$) while C14:1 tended ($P = 0.07$) to increase around parturition. Muscle carnitine remained unchanged, whereas those of C3:1, C3-DC, C3-OH, C5:1, C5-DC, C6:1, C12:1 were elevated ($P < 0.05$) around parturition. Consistent with the serum profile, we observed marked accumulation of long chain AC (C14:0, C14:1, C14:1-OH, C16, C16-OH, C16:1, C16:1-OH, C16:2-OH, C18:0, C18:1, and C18:2) concentrations in muscle around parturition which exhibited little or no change from d 21 to d 70. The CPT1 ratio (carnitine/C16:0+C18:0) decreased ($P < .0001$), whereas the CPT2 ratio (C16:0+C18:1/C2) increased ($P < .0008$) with the onset of lactation in both serum and muscle. The mRNA abundance of CPT1B increased 2.8-fold from d -21 to d 1 ($P = 0.02$), followed by a decline to nearly prepartum values by d 70. The mRNA abundance of CPT2 remained unchanged. The RQUICKI was numerically decreased from d -21 to d 1. Correlation analysis revealed a negative correlation between serum NEFA and CPT1 ratio ($P < .0001$; r = -0.66), and positive correlations ($P < .0001$) between serum NEFA and serum C12:0 (r = 0.53), C18:0 (r = 0.56), and C18:1 (r = 0.54), and muscle C6-OH (r = 0.50) and C16:0 (r = 0.55) across all time-points.

Conclusions: Muscle carnitine remained unchanged despite a decline in the serum concentrations, suggesting that the decrease in serum concentrations likely results from increased carnitine excretion in milk and increased carnitine uptake by the muscle to maintain the intracellular concentrations. The AC profiles demonstrated that the import of FA into muscle mitochondria shortly after parturition exceed muscle capability to appropriately oxidize FA. These data suggest that post-CPT1 events including deficiency or impaired function of CPT2 and depletion of several TCA cycle intermediates may result in accumulation of AC in skeletal muscle around parturition.

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132.

The effects of polyunsaturated fatty acids on markers of cartilage degradation in a canine model of osteoarthritis

Der Einfluss von mehrfach ungesättigten Fettsäuren auf Marker der Knorpeldegeneration in einem caninen Osteoarthritis-Modell

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Osteoarthritis (OA) is a common joint disease in dogs that causes chronic pain and stiffness. Studies indicate that an overproduction of interleukin-1β (IL-1β) leads to cartilage degeneration due to the activation of enzymes catalyzing the cleavage of matrix proteins. The destruction is further accelerated by inflammatory factors such as nitric oxide (NO) and prostaglandin E2 (PGE2). Treatment of OA is non-specific and limited to drugs with harmful side effects, such as NSAIDs and corticosteroids. Hence, new treatment options have to be considered. In order to evaluate potential beneficial effects of polyunsaturated fatty acids (PUFA) on cartilage degradation, we established a canine *in vitro* model using canine chondrocytes stimulated with IL-1β to mimic OA. Gene expression of enzymes involved in OA as well as inflammatory markers was determined in cells supplemented with n-3 or n-6 PUFA.

Methods: Canine chondrocytes were obtained from the knee joints of 6 healthy donor animals. Cells were cultivated until confluency in DMEM containing 10 % FBS, 1 % penicillin/streptomycin, 10 µg/ml insulin and 50 µg/ml phosphoascorbic acid. After supplementation with 10 µM arachidonic acid (AA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) for 6 days cells were stimulated with IL-1β for another 2 days. RNA was extracted from the cells and relative gene expression of catabolic and anabolic enzymes, including matrix metalloproteinase-3 (MMP-3), MMP-13, inducible nitric oxide synthase (iNOS), tissue inhibitor of metalloproteinase-2 (TIMP-2) and cyclooxigenase-2 (COX-2) was determined by real-time qPCR. Production of NO and PGE was measured in the cell culture supernatants by Griess reaction and immunoassay, respectively. Each experiment was carried out in four independent experiments performed at least in duplicate. Two-factorial ANOVA followed by Dunnett's post hoc test was used to compare differences between fatty acid supplements and IL-1β treatment. Significance level was set at $\alpha = 0.01$.

Results: Gas chromatography showed that all fatty acids were rapidly incorporated into the plasma membranes. Enrichment of the cells with PUFA increased MMP-3 gene expression. Furthermore, high levels of PGE were found after AA supplementation. In unsupplemented cells IL-1β treatment caused an up-regulation of gene expression for most inflammatory markers. NO and PGE levels were also significantly higher, compared to control cells. In cells supplemented with EPA or AA the mRNA levels of MMP-3 and iNOS were decreased and the production of NO was considerably attenuated. In contrast, expression of MMP-13 and release of PGE was elevated by both PUFA. The n-3 fatty acid DHA had only a marginal effect on the inflammatory markers.

Conclusions: Both AA and EPA may be beneficial for reducing cartilage degradation due to a diminished expression of inflammatory markers. Therefore, the ratio between n-3 and n-6 PUFA deserves attention in dietary interventions.

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133.

Fatty acid profile and flotillin-1 abundance in red blood cell membranes of dairy cows fed a total mixed ration with reduced essential fatty acid content

Fettsäureprofil und Flotillin-1 in der Erythrozytenplasmamembran bei Milchkühen mit reduziertem Gehalt an essentiellen Fettsäuren in der Ration

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Fatty acids (FA) are important components of the plasma membrane. Incorporated into the plasma membrane, they can influence the membrane fluidity and structure, which in turn have an impact on the function and abundance of membrane proteins. Flotillins are FA-anchored membrane proteins and markers of lipid rafts (1), a structural organization of the plasma membrane. These proteins are involved in a number of cellular processes, including signalling, endocytosis and trafficking. Common diets in dairy production are often based on corn silage that deliver lower amounts of n-3 FA like a-linolenic acid (ALA), compared to pasture based systems. This reduced intake of n-3 FA changes the n-6/n-3 ratio and may influence membrane composition of incorporated FA and thereby the abundance and function of membrane associated proteins. To verify this hypothesis, we investigated the fatty acid profile in red blood cell (RBC) membranes and analysed the amount of flotillin-1.

Methods: Five lactating cows (57 days in milk ± 12 d at start of the study) were investigated for 24 weeks after changing from a grass/corn silage based (GS) to a corn silage based TMR (CS) (2). Diets were isoenergetic (6.8 MJ NEL/kg of dry matter (DM)) and isonitrogenous (crude protein 155 g/kg DM), but crude fat content was lower in CS than in GS (22.38 versus 30.99 g/kg DM). Content of linoleic acid (LA) was quite similar (11.7 and 10.8 g/kg DM), but ALA content was much lower in CS than GS diet (1.0 versus 6.2 g/kg). From whole blood samples washed RBC were collected in week -1, 0, 1, 2, 8, 16, 24 and plasma membranes were isolated. Membrane samples were used for quantitative fatty acid analysis and to study flotillin-1 abundance by the Western blot method. Data were analysed by repeated measurement ANOVA using the MIXED procedure of SAS and results are presented as ± SEM. The model contained the fixed (repeated) effect time.

Results: The relative content of n-6 FA in RBC membranes increased with the CS diet (22.2 to 30.6 ± 1.7%; p

Conclusions: The diet low in n-3 FA modified the FA composition of RBC membranes and increased the n-6 to n-3 FA ratio. These changes were linked with an increase in the flotillin-1 abundance and might affect other membrane associated proteins in bovine RBC. Further studies are necessary to investigate possible effects on cellular processes.

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134.

Influence of conjugated linoleic acids (CLA) and vitamin E on milk fatty acid composition and α-tocopherol concentrations in blood and milk

Einfluss von konjugierten Linolsäuren (CLA) und Vitamin E auf die Fettsäurenzusammensetzung der Milch und die Konzentration von α-Tocopherol in Blut und Milch peripartaler Milchkühe

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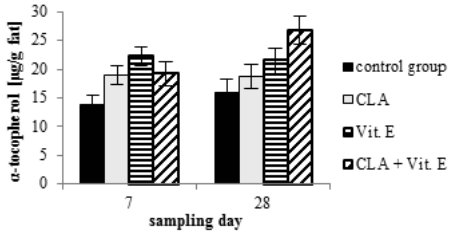
Different nutritional and technological properties of milk are influenced by its fatty acid composition. It has been observed that milk fatty acid composition can be altered by feeding individual fatty acids to cows. On the other hand vitamin E (Vit. E) as an antioxidant might also contribute to fatty acid stability and pattern. Thus, the aim of this experiment was to investigate effects of both CLA and Vit. E on fatty acid composition in milk and concentrations of vitamins closely associated to the oxidative status. Furthermore, we aimed to investigate the treatment effects on serum levels of α-tocopherol.

Methods: Fifty nine pluriparous German Holstein cows were allocated to four groups six weeks *ante partum* (a.p.). The CLA group (n=16) received 8.4 g *trans*-10, *cis*-12 CLA/d (BASF Lutrell®). The Vit. E group (n=15) received 2,327 IU vitamin E/d (BASF Lutavit® E 50), while the CLA + Vit. E (n=12) group got both supplements. The control group (n=16) as well as the Vit. E group got a control fat supplement for caloric balance. Milk samples were collected on days 7 and 28 *post partum* (p.p.) and analyzed for retinol, β-carotene, α-tocopherol and fatty acid composition. Blood samples were taken on days -42, -7, 1, 7, 14, 28 and 70 relative to parturition and were analyzed for cholesterol and α-tocopherol. Statistical analysis was performed by the MIXED procedure of the SAS software (9.4) for repeated measures with a compound symmetry structure. The factors in the model were treatment, time and the interaction between them. *P*-values < 0.05 were considered to indicate significant differences.

Results: No differences between treatments were found for retinol and β-carotene concentrations in milk. Concentration of α-tocopherol (µg/g fat) was higher in the Vit. E group compared to the control group on day 7 p.p. and higher in the CLA + Vit. E group on day 28 p.p. with a synergistic effect over pure Vit. E supplementation ($P < 0.05$) (Fig. 1). *Cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA were influenced by treatment with CLA. For *cis*-9, *trans*-11 CLA, differences between groups were not significant while percentages of *trans*-10, *cis*-12 CLA were higher in the CLA group compared to the control and the Vit. E group ($P_{Vit.. E x CLA} = 0.001$) on both sampling days. Percentage of *trans*-10, *cis*-12 CLA in the CLA + Vit. E group was enhanced on day 28 p.p. compared to the control group ($P < 0.05$). Ratios of α-tocopherol to cholesterol (Fig. 2) in blood serum were influenced by treatment with Vit. E ($P < 0.001$).



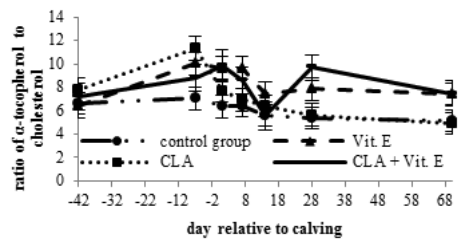


Fig.1 Concentrations of α-tocopherol in milk (LSMeans ± SEM) Fig.2 Ratios of α-tocopherol (mmol/L) to cholesterol (mmol/L) in blood serum (LSMeans ± SEM)

Conclusion: Treatment with CLA is suitable to enhance the proportion of CLA and Vit. E in milk. As treatment with Vit. E did not have an impact on milk fatty acid composition, it might be possible to change the oxidative status of the dairy cow without affecting milk properties.

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135.

Effects of abomasal infusion of essential fatty acids and conjugated linoleic acid on performance, fatty acid status and metabolism in dairy cows fed a ration with reduced essential fatty acid content

Einfluss einer abomasalen Infusion von essentiellen Fettsäuren und konjugierter Linolsäure auf die Leistung, den Fettsäurestatus und den Stoffwechsel bei Milchkühen mit einer reduzierten Versorgung an essentiellen Fettsäuren im Grundfutter

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Common diets in high-yielding dairy cows are mainly based on corn silage (CS), and provide low amounts of grass silage or fresh grass, resulting in low a-linolenic acid (ALA) supply. In a preliminary study we showed that feeding a diet based on CS reduces ALA and conjugated linoleic acid (CLA) content in milk and plasma, leading to a reduced status of ALA and CLA in lactating cows (1). The present study aimed to investigate the effects of the replenishment of essential fatty acids (EFA) and CLA in lactating dairy cows fed a CS based diet, and thus to detect their influences on performance, fatty acid status and metabolism.

Methods: Four rumen-fistulated lactating cows fitted with abomasal tubes (3rd lactation, 126 days in milk at start of the study) were investigated in a 4x4 Latin square design. The cows were fed a CS based total mixed ration (6.7 MJ NEL/kg of dry matter (DM)), providing 0.9 g/kg DM ALA and 9.9 g/kg DM linoleic acid (LA). Cows were daily supplemented either with coconut oil (CTRL, 38 g/d), linseed and safflower oil (EFA, 39 and 2 g/d), Lutalin® (CLA *c9, t11* and *c10, t12*, 5 g/d), and EFA+CLA. The initial dose was doubled twice for two weeks, resulting in a six week treatment period with three doses and followed by a 3-week wash out period. DM intake (DMI), milk yield and milk composition were measured weekly. Fatty acid composition in milk fat and blood plasma, plasma concentrations of metabolites and hormones (insulin-like growth factor binding proteins [IGFBP] only on wk 0 and 6) were analysed at wk 0, 2, 4, and 6 of each treatment period.Data were analysed by repeated measurement ANOVA using the MIXED procedure of SAS containing treatment, dose, and its interaction as fixed effects and week in milk as covariate.

Results: DMI was similar between groups, but milk yield was highest in EFA+CLA, and energy-corrected milk yield, milk fat content and milk yield declined in CLA and EFA+CLA in a dose-dependent manner (Pc9, t11 and *t10, c12* CLA increased (Pc9, t11 and *t10, c12*CLA increased in CLA and EFA+CLA in a dose-dependent manner (P

Conclusions: Supplementation of EFA and CLA or both led to an increase of the respective fatty acids in milk fat and blood plasma. CLA and EFA+CLA treatments resulted in increased citrate concentrations in milk and reduced urea concentrations in milk and blood, which indicated an effect of CLA on reduced milk fat synthesis and protein turnover. The lower IGFBP-2 in blood plasma might point at an improved energy status in EFA+CLA supplemented cows.

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136.

Are rumen-protected n-3 and n-6 fatty acids incorporated at different levels in bovine muscles with different metabolism?

Werden pansengeschützte n-3- und n-6-Fettsäuren in einem unterschiedlichen Ausmass in bovine Muskeln mit unterschiedlichem Stoffwechsel eingelagert?

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Recommendations for humans regarding the consumption of fatty acids (FA) suggest to increase intake of omega-3 (n-3) polyunsaturated fatty acids like eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), while maintaining or better lowering levels of omega-6 (n-6) polyunsaturated fatty acids. Unsaturated fatty acids in ruminant-source foods undergo an intensive biohydrogenation, where they become increasingly saturated, and thus, less desirable from a human health perspective. Novel coating techniques for the protection of unsaturated FA from microbial biohydrogenation now allow the targeted enrichment of ruminant-source foods with n-3 FA. As both, n-3 and n-6 FA, are functional FA, it is unclear whether they are enriched at the same level in all bovine muscles when supplemented in rumen-protected form. To clarify this aspect, beef heifers were fattened with such supplements and three muscles with different types and stages of involvement in locomotion and, therefore, different metabolism were analysed. One of the muscles selected, the *Extensor carpi radialis* (ECR), is very little examined so far and studying more than the commonly investigated muscles should provide a more differentiated view on metabolic physiology.

Methods: Thirty-three Angus heifers each received 7 kg dry matter/day of a standard diet composed of straw, hay, wheat, molasses, soybean meal in a ratio of 40:6:10:7:7 for 8 weeks until slaughter. This diet was supplemented daily with 0.45 kg coated lipids (Erbo Spraytec AG, Bützberg, Switzerland). Seventeen heifers received coated fish oil rich in n-3 FA (C3), the remaining 16 heifers were offered coated sunflower oil rich in n-6 FA (C6). The *Longissimus thoracis* (LT), the *Biceps femoris* (BF) and the ECR were sampled. Physicochemical meat quality and the profile of the intramuscular FA (IFA) were investigated. Data were analysed using the Mixed model of SAS (version 9.3) considering supplements, muscles and their interaction as fixed effects, and slaughter date as random effect.

Results: Supplement effects were mostly non-significant for meat quality parameters, such as muscle pH, colour, water-holding capacity, shear force and gross chemical composition. All muscles of the C3 heifers had a higher proportion (g/100 g total IFA) of n-3 IFA and a lower proportion of n-6 IFA compared to those of the C6 heifers (overall average n-3: 6.8 vs. 5.0, SEM: 0.54, $p<0.001$; n-6: 8.3 vs. 10.2, SEM: 0.89, $p<0.001$). Independent of the type of supplementation, there were differences between muscles in the level of IFA and their relative incorporation rate. In general, the ECR muscle had a higher proportion of n-3 and n-6 IFA compared to BF and LT (n-3: 9.5, 4.4, 3.7, SEM: 0.54, $p<0.001$; n-6: 14.8, 7.4, 5.4, SEM: 0.89, $p<0.001$). In addition, interactions ($P<0.01$) of supplement type and muscle were found for the fish oil specific long-chain n-3 FA, EPA and DHA, but not for total or most individual n-6 FA (except of C22:5n-6, a minor FA).

Conclusion: The results show that n-3 FA and n-6 FA are differently allocated to muscles. Further, the type of diet can influence the level of incorporation of individual n-3 FA. This suggests that there is a specific need for these FA which might be related to the intensity and type of locomotion these muscles are involved in. In addition, this means that, in order to profit from meat with particularly high n-3 FA proportions, distinct muscles have to be selected.

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137.

Role of glucose and acetic acid in differentiation of bovine pre-adipocytes into mature adipocytes

Bedeutung von Glukose und Essigsäure für die Differenzierung boviner Präadipozyten zu Adipozyten

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In mammals two types of stem cell are present either embryonic stem cells or adult stem. The adult mesenchymal stem cells either in bone marrow or other body fat depots include undifferentiated pre-adipocytes. Morphologically, pre-adipocytes resemble fibroblasts and can differentiate into adipocytes, chondrocytes and osteocytes. In monogastric animals, glucose is the main source of energy while in ruminants; short chain fatty acids (SCFA) serve the purpose. Among these SCFA produced by rumen fermentation, acetic acid is the main source of lipogenesis in ruminants (1). The aim of our study was to investigate the effects of acetic acid and glucose on the transformation of bovine subcutaneous pre-adipocytes into adipocytes.

Methods: Subcutaneous adipose tissue blocks (3 cm^3) were aseptically collected from calves (< 11 months old) to obtain bovine adipose-derived adult stem cells (ADAS). From the adipose tissue blocks, explant cultures of ADAS were obtained in DMEM:Ham's F12 (2) till passage four. After confluency of ADAS in 24-well culture plates, the cells were kept in induction medium containing: biotin (10 µM), pantothenic acid (10 µM), insulin (3 µg/ml), dexamethasone (0.3 µM), IBMX (0.1 µM) and rosiglitazone (10 µM) for 2 d. Subsequently, cells were kept in complete base medium (DMEM:Ham's F12, without fetal bovine serum) with 10 mM glucose and 0 mM acetic acid (TRT-1), 10 mM glucose and 10 mM acetic acid (TRT-2), 10 mM glucose and 20 mM acetic acid (TRT-3), 25 mM glucose and 0 mM acetic acid (TRT-4), 25 mM glucose and 10 mM acetic acid (TRT-5), 25 mM glucose and 20 mM acetic acid (TRT-6). After the 2-d induction period, cells were kept in six differentiation media either for 14 or 28 d. For the first 2 d in either differentiation medium, cells were stimulated with 5 µl/ml of bovine serum lipids (Ex-Cyte). After 14 or 28 d, cells were processed for estimation of neutral lipids by Nile red stain and for GPDH production. Photometric quantification of Nile red was used to monitor lipid formation. Enzymatic activity of GPDH in cell lysates was measured at DA_{340} and at 30°C according to the method of Grant et al. (3). Statistics was conducted by two-way ANOVA and Holm-Sidak method, using the SigmaPlot 11.0 software package.

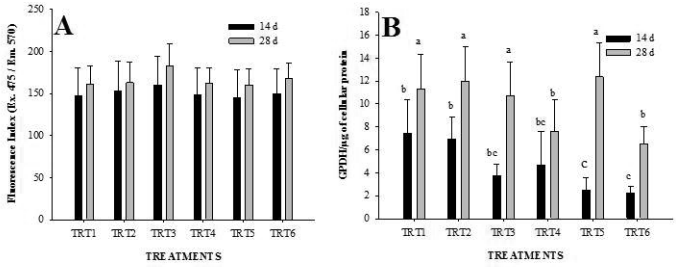

Figure 1: Adipocyte non-polar lipids concentration (A), development of glycerol-3-phosphate dehydrogenase (GPDH) in adipocytes (B) when co-incubated in different concentrations of acetic acid and glucose. [a–c]Least squares means without a common superscript letter differ ($P < 0.05$).

Results: The results showed the conversion of ADAS by formation of lipid droplets in all treatments. As indicated in Figure 1A, the addition of acetic acid (20 mM) in medium enhanced the accumulation of lipids when glucose concentration was low (10 mM) as compared to control and high glucose concentration (25 mM); however, this was not statistically significant. The activity of GPDH was significantly higher ($P <$ 0.05) in all groups after 28 days of incubation in the differentiation medium (Figure 1B). With low glucose concentration the activity of GPDH also remained higher as comparted to high glucose concentration in TRT-4, TRT-5 and TRT-6.

Conclusion: The applied experimental protocol successfully induced differentiation of ADAS. There is a weak indication that ADAS maturation into adipocytes can be promoted by acetic acid; however, this requires further investigations.

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138.

Effect of dietary conjugated linoleic acid on vitamin A status of lactating rats and their offspring

Effekte konjugierter Linolsäuren auf den Vitamin A Status von laktierenden Ratten und deren Nachkommen

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Conjugated linoleic acid (CLA) is known to affect the lipid metabolism in animals and humans (1), and the absorption and metabolism of vitamin A and of lipids is interlinked (2). However, effects of CLA on the metabolism of fat-soluble vitamins in lactating animals and co-occurring effects on their offspring are unknown. Therefore, the aim of this study was to investigate effects of dietary CLA on concentrations of retinol in tissues of lactating rats and their offspring and to determine expression of genes involved in retinoid metabolism and transport in dams.

Methods: Twenty-eight female Wistar Han rats were allocated to 2 groups and fed a control diet (control group) or the same diet with 1.5% of sunflower oil replaced by a CLA preparation (Lutalin, BASF) which supplied each 0.45% of cis-9, trans-11 and trans-10, cis-12 CLA to the diet (CLA group) during pregnancy and the first 14 days of lactation. Feed intake of dams and body weight of dams and their pups were recorded weekly. In accordance with the German Animal Welfare Law, dams and pups were sacrificed (JLU No. 480_M) and samples of lung, stomach contents (pups only), plasma, gastrocnemius muscle (dams only) and of liver and adipose tissue were collected, snap-frozen in liquid N_2 and stored at -80°C. Plasma and tissue retinol concentrations were determined 14 days after parturition in dams and 1, 7 and 14 days after birth in pups by HPLC (3) and excitation and emission wavelengths of 325 and 475 nm, respectively. Gene expression of CYP26A1, a cytochrome P450 enzyme involved in retinoid metabolism, cellular retinol binding protein 1 (CRBP1), lecithin: retinol acyltransferase (LRAT), retinol binding protein 4 (RBP4), and of transthyretin (TTR) was determined in dam liver, and of CRBP1, LRAT, cellular retinoic acid binding protein 1 (CRABP1), and "stimulated by retinoic acid 6" receptor (STRA6) in dam adipose tissue by real-time PCR according to Zeitz et al. (3). The data were statistically analysed by analysis of variance considering treatment and, if applicable, time and the interaction of treatment and time as fixed effects, using the GLM procedure in Minitab13.

Results: Feed intake and body weights of pregnant and lactating dams, and body weights of pups at birth and 7 and 14 days after birth, were similar in both groups. Retinol concentrations in dam plasma (Control: 345±54 and CLA: 339±76 ng/ml), muscle (31.9±5.3 and 31.9±6.6 ng/g) and liver (184±36 and 183±47 µg/g) were similar (P>0.10). Likewise, relative mRNA concentrations of CYP26A1, CRBP1, LRAT, RBP4 and TTR in liver and of CRBP1, LRAT and STRA6 in the adipose tissue were similar between groups, indicating that retinol transport from liver to adipose tissue via the RBP4-STRA6 system was not affected by CLA feeding. However, relative mRNA abundance of the low density lipoprotein (LDL) receptor (LDLR) which is involved in extrahepatic uptake of retinoids from LDL, was increased by 1.5-fold (P = 0.041) which may explain why adipose tissue retinol concentrations were in tendency higher in CLA-fed dams (965±185 ng/g) compared to control dams (847±101 ng/g) (P=0.063). In addition, relative mRNA concentrations of CRABP1 in the adipose tissue were 1.7-fold higher in CLA-fed dams compared to control dams (P = 0.079). In the milk curd removed from the pup´s stomachs, and in liver, lung and adipose tissue of pups, retinol concentrations at days 1, 7, and 14 were similar in both groups.

Conclusion: We show that dietary CLA slightly affect adipose tissue retinol concentrations in lactating rats, which may be explained by increased uptake of retinoids from LDL via the LDLR, but had no effect on tissue retinol concentrations in their offspring. These data indicate that moderate dosages of dietary CLA in pregnant and lactating animals and humans may be uncritical considering the retinol status of new-borns.

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139.

Palmitoylethanolamide -a natural agent with possible anti-inflammatory and anti-oxidative effects against canine atopic dermatitis

Palmitoylethanolamid - ein natürliches Mittel mit möglichen entzündungshemmenden und antioxidativen Wirkungen gegen atopische Dermatitis

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Mast cells are implicated in a variety of allergy and autoimmune disorders such as canine atopic dermatitis (CAD). It is thought to be the major effective cells in the pathogenesis of CAD through the release of a variety of inflammatory mediators which aggravate the clinical picture of the disease. Moreover, enhanced oxidative stress contributes to mast cell dysfunction via apoptotic induction. Palmitoylethanolamide (PEA) and related lipid mediators are endogenous bioactive compounds, considered to play a protective role in many tissues. Evidence collected so far shows that the anti-inflammatory effects of PEA depend on the down-modulation of mast cell degranulation. The goal of this work was to investigate the effects of PEA on mast cell functions such as histamine release and ß-hexosaminidase activity. Moreover, the effect of PEA on the generation of intracellular reactive oxygen species (ROS) was investigated.

Methods: Canine mastocytoma cells (C2) were cultured in a basic medium or in media supplemented with 1, 5, 10, 15 or 20 µmol/l of PEA for 4 days and stimulated with 25 µmol mastoparan as a model for CAD. Histamine release and ß-hexosaminidase activity were measured by HPLC and spectrophotometry, respectively. ROS production was assessed with the oxidation-sensitive fluorescent probe 2′, 7′-dichlorofluorescein. Results were analyzed using the Student's *t*-test for unpaired data. A probability level of 0.05 or smaller was used for statistical significance. Results were expressed as the mean ± SD. The statistical analysis was carried out with GraphPad Prism 4 (GaphPad Software, La Jolla, CA, USA).

Results: PEA did non affect levels of histamine, ß-hexosamindase activity and ROS production in unstimulated C2. In mastoparan-stimulated cells the histamine release decreased significantly to 12, 36, 45, 55 and 61% in a dose dependent manner (Fig. 1). ß-hexosaminidase activity in mastoparan-stimulated cells decreased similarly to 1, 1, 16, 18, and 22%, compared to stimulated control cells. On the other hand, PEA exposure diminished ROS production in mastoparan-stimulated C2 significantly to 33, 37, 47, 48, and 49%, respectively (Fig. 2).

Conclusion: In this study we demonstrate that PEA modulates inflammatory mediators released by stimulated mast cells, thus driving the immune response in an anti-inflammatory direction. Therefore PEA may have beneficial effects in inflammatory diseases such as atopic dermatitis.

Figure 1 Effect of PEA on histamine release

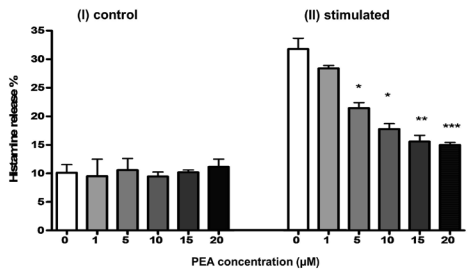
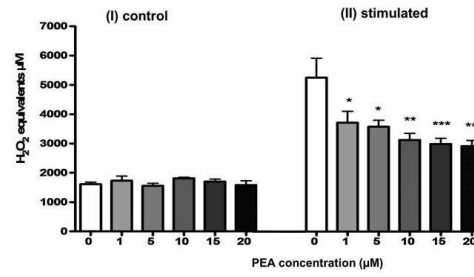


Figure 2 Effect of PEA on ROS production



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140.

Antioxidant impact and ovary Δ-6 desaturase gene expression during pregnancy, milk fatty acid profile in rabbit does fed with fatty acids supplementation

Antioxidative Wirkung und Expression der Eierstock Δ-6-Desaturase während der Trächtigkeit, Milchfettsäuren-Profil in Zuchtkaninchen gefüttert mit ergänzten Fettsäuren

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Introduction: Contrasting results have been obtained regarding the effect of supplementation with polyunsatured fatty acids (n-3 PUFA) in animal metabolic and reproductive processes (1).
Long chain n-3 and n-6 PUFA (LCP) have specific and important health benefits due to antinflammatory, hypolipidemic, and antioxidant effects in animals and humans who consume animal products. These fatty acids can be obtained as such from the diet or synthesized endogenously from PUFA precursors via Δ-5 and Δ-6 desaturases, encoded by FADS1 and FADS2, respectively. Addition of eicosapentanoic (EPA; C20:5 n-3) and docosahexaneoic acids (DHA; C22:6 n-3) present in fish oils into the diet of rabbit females, provides a direct intake of LCP, and are involved in a correct development of pregnancy and lactation in rabbit does, as PUFA are implicated in both prostaglandins and steroid metabolism (2). Flaxseeds are a rich source of ALA (a-linolenic acid; C18:3 n-3) which is a precursor of EPA and DHA through the complex and limiting step mechanism of elongation and desaturation involving Δ-6 desaturase enzyme. For this reason, FADS2 may be determinant in the effects induced by EPA and DHA on the animal reproduction and metabolic activity (3). Furthermore, FADS genes are strongly associated with reproduction and lipid metabolism function (4). The objective of this work was to evaluate the effects of maternal supplementation with fish oil and extruded linseed on some parameters of reproductive performance and of its metabolism.

Methods: Thirty nulliparous New Zealand White rabbit does were randomly subdivided in three groups and fed either a control diet (C group, n=10), an iso-energetic diet supplemented with 3% fish oil (Nordos ® - FO group, n=10) or an iso-energetic diet supplemented with 10% of extruded linseed (L group, n=10). Nutritional treatment of does begun 3 months before insemination and continued until weaning (35th day post-partum). Blood samples were obtained before treatment, 3 months after administration of different diets, and at 28 days of pregnancy. Ovaries were collected at day 28 of pregnancy and in the post-weaning period at day 20. To evaluate the expression of FADS2 by RT-PCR, total RNA was extracted from 3 rabbit ovaries per group and physiological period examined. From day 1 to day 20 of age, BW and milk intake of the litter were recorded daily. Concentrations of plasma thiols (Cys, Hcy and GSH) were determined using HPLC with reverse-phase separation and fluorescence detection (λex 385 nm and λem 515 nm). The fatty acid profile of milk was determined by gas chromatography. Individual fatty acids methyl esters (FAMEs) were identified using analytical standards (PUFA-2) and quantified using nonadecanoic acid methyl ester (C19:0) as internal standard.

Results: There were no differences in BW or milk intake between the three different litter groups. However, the dietary supplementation of n-3 FA affected the FA profile of milk, increasing the PUFA n-3 content respect to the control. The FADS2 gene was highly expressed in the ovary, but there were no significantly differences between dietary groups. Over time concentrations of thiols tended to drop expecially in the FO and L groups.

Conclusions: The ovarian tissue of does fed n-3 enriched diets seems to be involved in the modulation of lipid metabolism, as evaluated by the presence of FADS2 mRNA. Further studies are needed to clarify the degree of involvement of this tissue and the effects of such dietary interventions on the offspring metabolism. The effects of supplementation of the maternal diet with FA may be important even in the offspring since it increases the levels of PUFA in milk.

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141.

Investigations regarding the localisation (anatomical parts) of small intestine elongation in case of exocrine pancreatic insufficiency (EPI) in pigs

Untersuchungen zur Beteiligung verschiedener anatomischer Abschnitte im Falle der Verlängerung des Dünndarmes bei Vorliegen einer exokrinen Pankreasinsuffizienz (EPI) von Schweinen

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Exocrine pancreatic insufficiency (EPI) in pigs can be induced by pancreatic duct ligation (PL) allowing to use these animals as a model for EPI in humans. In former studies [1,2] an elongation of the small intestine (SI) in EPI-pigs up to 6 meters within 10 weeks was observed. Due to sampling procedures in these former studies information is lacking, whether all regions of the SI are involved in parallel or whether some segments are involved in this process prominently. Therefore this study aimed to determine, which anatomical parts of the SI are taking part in the process of SI elongation. As visceral smooth muscle cells are considered to be postmitotic cells, although mitosis in matured smooth muscle cells can occur [3], the hypothesis of this study was that SI elongation is associated with changes in histological morphology.

Materials and Methods: The tissue samples derived from cross-bred female pigs (n=8) euthanised at the age of 26 weeks. In 5 pigs EPI was experimentally induced by PL at the age of 16 weeks, while a sham-operation was performed in control pigs (C) at same age. All animals were fed a complete diet. During dissection the duodenum, jejunum and ileum were separated according to macroscopical landmarks. Tissue samples for histological investigations were taken from the beginning, 25 %, 50 %, 75 % and at the end of the jejunum and embedded in Technovit®. Sections were stained with toluidine blue and the number of cell nuclei was counted using an area of 40 x 40 µm.

Results: PL resulted in a significant increase in the length of the small intestine (see table 1). While the length of jejunum was significantly increased, the length (cm) of duodenum (PL: 56.7 ± 11.1; C: 48.3 ± 7.02) and ileum (PL: 53.4 ± 13.7; C: 45.6 ± 5.50) did not differ ($p>0.05$) between groups as well as the relative weight of SI tissue (see table 1). The number of cell nuclei was significantly ($p<0.05$) higher in the tunica muscularis of the jejunum in C-pigs (see table 1).

Table 1**:** Length of the small intestine and the jejunum, relative weight of emptied small intestine (kg/meter tissue) and number of cell nuclei in the tunica muscularis of the jejunum

Parameter	Control (n=3)			PL (n=5)		
Body weight at slaughtering (kg)	146^a	±	1.00	111^b	±	8.07
Length of the small intestine (m)*	20.7^a	±	1.89	25.8^b	±	1.59
Length of jejunum (m)	19.7^a	±	1.94	24.7^b	±	1.57
Mass of small intestine (kg fresh weight)	1878^a	±	456	2407^b	±	281
Mass of SI (kg fresh weight/m length)	0.092^a	±	0.016	0.093^a	±	0.009
Number of cell nuclei in the tunica muscularis of the jejunum (area of 40 x 40 µm)						
Stratum circulare (tranverse cut)	3.71^a	±	2.17	2.20^b	±	1.53
Stratum longitudinale (transverse cut)	1.88^a	±	1.47	1.10^b	±	0.980

Different letters (a,b) mark significant ($p<0.05$) effects of group (Fischer's LSD)

Discussion: The earlier finding of EPI induced elongation of SI was confirmed (5 meter within 10 weeks). The elongation is based on reaction of jejunum - which is not surprising as this is by far the longest segment of the SI of pigs. The fact that there was a higher SI mass in PL 16 and no difference in relative weight of the tissue demonstrates that elongation is *not* caused by stretching or thinning of the SI. The lower number of cell nuclei in the tunica muscularis indicates a hypertrophy of smooth muscle cells (volume of each singular muscle cell ↑; no increased proliferation rate) in PL-pigs.

Conclusion: The jejunum is the prominent part of SI showing elongation due to induced EPI. Although relative mass of tissue is not affected there are changes of the tunica muscularis regarding histological morphology in the elongated SI of PL-pigs - indicating adaptation in postmitotic cells.

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142.

Longitudinal analysis of faecal microbiome composition of sows after offering a high fibre supplement (TFM) around parturition

Verlaufsanalysen zur Zusammensetzung des Mikrobioms im Kot von Sauen nach dem Angebot eines rohfaserreichen Supplements (TFM) um die Geburt

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High fibre diets offered to periparturient sows are proposed to have different beneficial effects (Oliviero et al., 2009). The aim of this study was to investigate the influence of a high fibre supplement (TFM) offered in addition to a commercial lactation diet on the composition of the faecal microbiome. Samples were obtained before the start of the experiment, while feeding the TFM and after returning to a control diet to test immediate and lasting changes using 16S rRNA amplicon sequencing.

Material and Methods: In total 25 sows were fed a commercial lactation diet (2.5 kg DM/d + 0.4 kg DM/d/piglet) restrictively. 12 of 25 sows were offered high fibre pellets (TFM) *ad libitum* out of an extra feed dispenser from d 5-7 a. p. until d 1-2 p. p.. To improve the palatability of TFM-pellets, these were mixed with 15 % of a commercial lactation diet. From d 3 p. p. onwards lactation diet was filled into the extra feeding dispensers. The fibre pellets consisted mainly of barley, hydrolysed soybean hulls and oat bran (per kg DM: 125 g XP, 179 g XF; 10.0 MJ ME). Microbiome composition was analysed from faeces of all sows at three time-points (d 7 a. p., d 1-2 p. p. and d 15-17 p. p.) using 16S rRNA analysis (Miseq, Illumina). Statistical analyses were done by using the SAS software (analysis of variance and Wilcoxon-Two-Sample-Test, respectively, $p < 0.05$).

Results and Discussion: The daily voluntary intake of the high fibre supplement was 3.14±0.68 kg DM. Before the start of the study on d 5-7 a. p., no major differences in faecal microbiome composition could be observed between the two groups, except minor changes in *Spirochaetales* and *Christensenellaceae.* The order with the highest relative abundance in both groups was *Clostridiales* (about 50%). On d 1-2 p. p. increased relative abundances of *Lactobacillaceae* and *Lachnospiraceae* were observed in the TFM group, while *Ruminococcaceae* and *Christensellenaceae* were decreased. On d 15-17 p. p. no significant differences were seen between both groups except in the order *RF39,* which generally has a low abundance. *Lactobacillales and Clostridiales* observed over all 3 points of time are shown in tab.1.

Tab. 1: Mean value ($\bar{x}$) and standard deviation (s) of Lactobacillales and Lactobacillaceae in the faeces (relative abundance %)

		d 5-7 a. p.			d 1-2 p. p.			d 15-17 p. p.		
	Group	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	S
Lactobacillales	C G	12	20.0^{a}	±12.2	12	8.23^{b}	±5.60	13	8.99^{b}	±10.6
	TFM	11	16.1^{ab}	±9.32	12	19.6^{a}	±8.71	10	8.63^{b}	±7.79
Clostridiales	C G	12	50.5^{a}	±10.5	12	55.8^{ab}	±7.46	13	59.5^{b}	±12.6
	TFM	11	49.7^{ab}	±9.66	12	44.1^{a}	±10.3	10	62.6^{b}	±14.5

^{a,b} different super-scripts within a row indicate significantly different values ($p < 0.05$) C G = Control Group, TFM = high fibre diet, a. p. = ante partum, p. p. = post partum

Comparing the microbiome at d 5-7 a. p. to d 15-17 p. p. revealed increased abundances of *Clostridiales* and *Clostridiaceae* and decreases in *Lactobacillaceae* and *Lactobacillales* in both groups. Relative abundances of *Ruminococcaceae* were not changed. The additional offering of lactation diet *ad libitum* out of the feed dispenser instead of TFM up to d 3 p. p. did not have a significant influence on the faecal microbiome.

Conclusion: The composition of the faecal microbiome in sows was significantly influenced within a short period of only 6-9 days by feeding TFM; especially concerning an markedly increase of *Lactobacillaceae* but no lasting effects after removal of the diet were observed. Increases in *Lactobacillaceae* might have practical relevance and may benefit the newborns.

LIVIERO et al. (2009): Res. Vet. Sci. 86: 314-319

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143.

Fattening performance of dual purpose cockerels as compared to an extensive broiler genotype and laying hybrid cockerels when fed a low protein diet

Leistung von Zweinutzungshähnen gegenüber einem extensiven Broilergenotyp und Legehybridhähnen beim Einsatz eines proteinarmen Futters

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The extreme specialisation in poultry production towards egg or meat production resulted in the practice that 1-day old male layer cockerels are culled at hatch. Instead, dual purpose genotypes could be used, where females are suitable for egg production and males for meat production. However, a lower performance and feed efficiency compared to the specialised types is to be expected. Therefore, they are particularly suitable for organic production systems which do not aim at maximum performance. About one-third of the typical organic broiler diet consists of soybean components (mostly cake where no solvents are used in processing) because a rather high dietary content of essential amino acids (especially lysine) is needed to avoid supplementation of synthetic amino acids. As the use of soybean in animal nutrition is controversially discussed, the aim of the present study was to determine whether dual purpose genotypes would tolerate a diet lower in soybean products and protein better in general than extensive broiler genotypes.

Methods: A dual purpose genotype (Lohmann Dual cockerels, LD; n = 24), an extensive broiler (Hubbard JA 957, both genders, HU; n = 28) and a layer hybrid (Lohmann Brown cockerels, LB; n = 24) were compared during 9 weeks of fattening. The birds were kept in pairs in cages of 0.64 m^2. They were fed *ad libitum* and received either a control diet (C) or a protein reduced diet (-P), where the proportion of soybean cake was reduced from 30 in C to 15%, maize gluten (5% in C) was omitted and compensation was done with cereals, rapeseed cake and sunflower cake. The two diets contained the same amount of energy (12.6 to 12.7 MJ/kg ME, but the CP content was reduced from 215 g/kg (C) to 167 g/kg as fed). Body weight (BW) and feed intake were determined weekly. At slaughter, carcass and breast meat were weighed. Data were subjected to ANOVA using SAS 9.3 and were analysed considering genotype, diet and interaction as fixed effects. For multiple comparisons of the Least Square means the Tukey-Kramer option was used. Statistical significance was set to $p < 0.05$.

Results: Final body weights (BW), carcass weights and average daily gains (ADG) differed ($p < 0.05$) between diet types and between genotypes (Table 1). With diet -P, the largest growth depression was found with HU, whereas growth levels were only numerically lower in the LD and the LB cockerels. Still the proportionate decrease (e.g. in final BW from C to -P by 11% and 8%) was similar, but was more pronounced with 16% in the layer cockerels. Across genotypes, feed conversion ratio (FCR) was more favourable with C than -P. Breast meat proportion (BP) was not influenced by diet type.

Table 1: Diet and genotype effects on performance

Genotype	Hubbard		Lohmann Dual		Lohmann Brown		
Diet	C	-P	C	-P	C	-P	SEM
BW (g)	3005^{a}	2662^{b}	2159^{c}	1997^{c}	1273^{d}	1066^{d}	93.0
Carcass weight (g)	2094^{a}	1829^{b}	1374^{c}	1265^{c}	769^{d}	634^{d}	66.1
ADG (g/d)	47^{a}	42^{b}	34^{c}	31^{c}	20^{d}	16^{d}	1.5
FCR (g feed/g ADG)	2.04^{b}	2.25^{b}	2.27^{b}	2.55^{ab}	2.43^{ab}	2.81^{a}	0.162
BP (% of carcass)	26.0^{a}	24.6^{a}	20.1^{b}	18.6^{bc}	16.2^{c}	16.6^{c}	1.99

$^{a-d}$Means within a row carrying no common superscript are significantly different ($p < 0.05$).

Conclusion: Reducing dietary CP to 167 g/kg was too far reaching to prevent a growth depression in the LD genotype, even though the absolute depression was lower than that found in the extensive broiler type used in organic farms. Future studies therefore have to determine the real protein and amino acid requirements of dual purpose genotypes to be able to minimize the use of potential human foods in the diet.

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144.

Effect of floor design (with/without litter) in broiler and turkey housings on feed intake, body weight and foot-pad health

Einfluss der Bodengestaltung (mit/ohne Einstreu) bei Broilern und Puten auf die Futteraufnahme sowie die Entwicklung von Körpermasse und Fußballengesundheit

*Chuppava B., Visscher C., Ratert C., Keller B., Kriewitz J.- P., Kamphues J. – Hanover

In Europe housing of poultry on littered concrete floor is the most common form. At the end of the fattening period more than 95 % of the dry matter consist of excreta [1]. In fattening of turkeys renewing of litter material is in fact practiced. The continuous contact of birds' feet to wet excreta is a predisposing factor for development foot-pad dermatitis. The hypothesis of these ongoing experiments is that 'separation' of animals from parts or at highest degree from their excreta decouples the associations between performance and foot-pad health.

Methods: Three consecutives trials with 240 chickens (Ross 308) each and two consecutives trials with 240 turkeys (Big 6) were performed (third is still running). After seven days of rearing in large groups, animals were randomly assigned to four groups with three subgroups each. Number of animals was reduced by dissection on day 22 to 144 animals in every trial. Different floor designs were created and installed to enable differently intense contact of the animals to the mixture of litter and excreta: G1 - entire floor pens with litter, G2 - floor pens with litter and heating pad, G3 - partially (50:50) slatted floors including an area that was littered, G4 - fully slatted floors with a sand bath (900 cm^2). Feed intake was recorded daily. The development of body masses and the evaluation of the foot-pad health ([2]; 0 = normal skin; 7 = > half of foot-pad necrotic) was carried out once a week. Statistical analysis was performed with SAS. To compare the feed intake, body mass and foot-pad scores Kruskal-Wallis- and Wilcoxon-tests as well as Spearman Correlation ($p < 0.05$) was used. Comparison on all parameters took place on average data of the subgroup.

Results: For broilers the average body weight of groups with a partly or fully slatted floor was significantly higher. In turkeys, foot-pad scores were significantly lower when using a slatted floor. For broilers, a significantly high correlation between body mass and foot-pad health could be shown for littered systems. The level of the foot-pad scores at all was very low. In turkeys, the correlations for the identical systems were significantly negative. With slatted systems a correlation between body mass and foot-pad health did not occure.

Table 1: Average feed intake, body weight, foot-pad scores (FPD-scores) and Spearman correlations between performance parameters and foot-pad health in consecutive trials with broilers and turkeys (G1: litter; G2: litter with heating pad; G3: partly slatted floor; G4: fully slatted floor)

Species	Group	Ø feed intake/animal	Ø body weight d 36	FPD-Score	Spearman A vs C		B vs C	
		A	B	C	r	p	r	p
Broiler (n=720; n=429***)	G1	3604±97.8[a]	2555±116[b]	0.40±0.24[a]	0.28	0.47	0.73	0.03
	G2*	3624±118[a]	2569±67.3[b]	0.45±0.26[a]	0.37	0.33	0.80	0.01
	G3	3663±61.5[a]	2655±64.2[a]	0.61±0.16[a]	0.74	0.02	0.34	0.37
	G4**	3698±145[a]	2698±123[a]	0.59±0.27[a]	0.33	0.38	0.38	0.31
Turkey (n=480; n=288***)	G1	2683±212[a]	1995±139[a]	4.30±0.46[a]	-0.70	0.12	-0.81	0.05
	G2	2650±258[a]	1962±187[a]	4.10±0.33[a]	-0.83	0.04	-0.83	0.04
	G3	2757±161[a]	2007±105[a]	4.10±0.16[a]	0.60	0.21	0.30	0.21
	G4	2872±200[a]	2093±112[a]	3.34±0.45[b]	0.09	0.87	0.26	0.62

[a, b, c] averages differ significantly within a row on species level ($p < 0.05$), * two animals died, **one animal died; *** number of animals after dissection.

Conclusion: The numerically higher feed intake resulted in higher final weights overall. Perhaps the limited possibility of pecking in the litter might result in a higher feed intake of the animals. Keeping of animals on partly or fully slatted floors decouples associations between litter quality, performance and foot-pad health.

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145.

Ingestive mastication in horses parallels rumination but not ingestive mastication in cattle and camels

Das Kauen von Pferden entspricht dem Wiederkauen, nicht dem Fresskauen von Rindern und Trampeltieren

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A comparison of the particle size in the faeces of horses and the forestomach contents of ruminats suggests that equid ingestive mastication is more thorough than that of ruminants. This could e.g. be due to a higher chewing intensity (per g food), due to a more efficient dental design, or differences in the mastication patterns. Although a large number of studies on chewing in ruminants (1) or equids (2) exist, direct comparisons of chewing measurements between these groups are rare (3). In order to characterize mastication in horses and two ruminating species, we used a validated chewing halter system (RumiWatch, Itin + Hoch GmbH, Liestal, Switzerland; 4,5).

Methods: 6 individual horses (563 ±44 kg), heifers (459 ±110 kg) and Bactrian camels (645 ±60 kg) were fitted with the same RumiWatch chewing halters that record mandibular movements using a pressure sensor and a proprietary algorithm that was developed to classify chewing events of cattle as 'ingestive' or 'rumination' mastication. Software custom-supplied by Itin + Hoch facilitated the measurement of individual chewing peak intervals (PI), peak heights (PH), and peak breadths (PB). All animals were offered grass hay of the same batch (74 g crude protein and 607 g neutral detergent fibre per kg dry matter) for 15 minutes; in cattle and camelids, measurements were continued subsequently until rumination was observed (except for one camel that did not ruminate within 2 h). In each individual, 10 subsequent chewing bouts (of ingestion and - in ruminanting species - rumination) were used to calculate the standard deviation (SD) of PI, PH and PB as proxies for the regularity of the chewing pattern. Because PH is sensor-dependent, it can only be compared within an individual. After confirming normal distribution, data were analysed by paired t-test (comparing ingestive and rumination mastication within species) ANOVA and Sidak post hoc tests between species.

Results: Dry matter intake averaged 3.5, 2.5 and 1.9 g/kg$^{0.85}$ in horses, cattle and camels. In cattle and camelids, ingestive mastication was properly identified in 85 and 87% of observations, and rumination mastication in 95% of observations. In both ruminating species, ingestive mastication was less regular than rumination, indicated by significantly higher SD for PI, PH and PB in intra-individual comparisons. With the exception of one horse, 96 ±3 % of horse ingestive mastication was identified as 'rumination' by the proprietary algorithm. The mean SD of both PI and PB in horses (0.08 ±0.06 and 0.05 ±0.01 sec, respectively) was significantly lower than ingestive PI (0.19 ±0.05 and 0.24 ±0.07 sec) and PB (0.07 ±0.01 sec in both cases) of cattle and camels. In contrast, the horse mean SD did not differ significantly from rumination PI (0.07 ±0.02 and 0.12 ±0.03 sec) and PB (0.05 ±0.00 and 0.04 ±0.01 sec) of cattle and camels, suggesting equally regular chewing motions

Conclusion: The results have to be interpreted with caution because satiation state, and hence ingestion voracity, might have differed between individuals and also between species, as they were kept in different husbandry systems, which might have influenced the measurements. Nevertheless, the results indicate that ingestive mastication of horses is characterised by consistently regular chewing motions typically associated with, and observed in, ruminating ruminants. Regular, rhythmic chewing hence represents a common feature of these distantly related species. Given this similarity, it appears that less consistent or irregular ingestive mastication in ruminants is a deviating pattern whose adaptive value remains unclear; in particular, it does not appear to be linked to a faster ingestive processing of food. A potential ultimate cause may be the avoidance of high tooth wear rates by adjusting ingestive mastication to the actual food bolus, and delaying a more regular, systematic mastication until after the ingesta has been softened and washed in the forestomach.

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146.

Comparative effects of lipopolysaccharide challenge on cytokine mRNA expression in the liver in non-obese ponies and horses

Vergleichende Effekte einer Lipopolysaccharid Provokation auf die mRNA Expression von Zytokinen in der Leber von normalgewichtigen Ponys und Pferden

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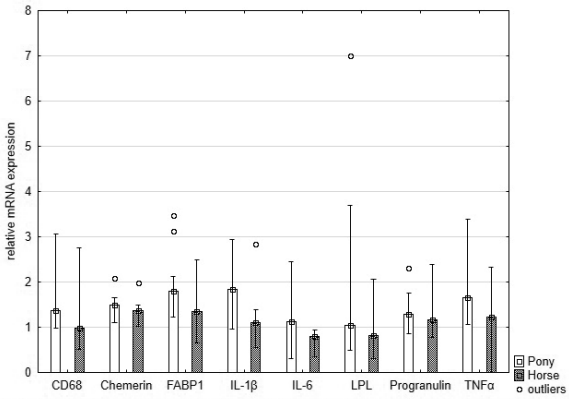
Question: The role of the liver in the metabolic syndrome has not been investigated intensively in horses and ponies. Furthermore the reasons for the higher predisposition of ponies for metabolic disorders have not been identified yet. The aim of the study (No. TVV 32/15) was to investigate the different hepatic reactions between ponies and horses to a lipopolysaccharide (LPS) challenge. We hypothesized that already in lean body condition ponies react with a more profound inflammation response to the LPS challenge compared to horses.

Methods: 10 Shetland ponies (age 6±3 years, mean body weight ± SD: 118±29 kg) and 10 warmblood horses (age 10±2.6 years, mean body weight ± SD: 589±58 kg) were included in this study. Animals were all non-obese with a median (25./75.percentile) body condition score of 3.65 (2.15/4.4) for the ponies and 3.57 (3.05/4.2) for the horses on a scale from 1-6. All animals were considered healthy, insulin sensitive and without a history of metabolic disorders. LPS was given as a challenge of 10ng/kg body weight. 15 hours after the LPS challenge liver tissue samples were taken under general anesthesia. Specimens of liver were analyzed by RT-qPCR for mRNA expression of IL-6, TNFα, CD68, chemerin, FABP1, LPL, progranulin and IL-1β. HPRT1, RPL32 and 18S were selected as reference genes. Serum was collected before and 14 hours after LPS challenge to analyse serum amyloid A (SAA). Data were assessed for normality by the Shapiro-Wilk test. mRNA expression and SAA were subjected to the Mann-Whitney U-test to compare ponies with horses. Wilcoxon test was performed to compare SAA before and after LPS challenge. Statistical significance was accepted at $P<0.05$.

Results: SAA values were significantly higher for ponies and horses after LPS challenge compared to baseline values. No statistically significant differences were noted in mRNA expression of CD68, chemerin, FABP1, IL-1β, IL6, LPL, progranulin and TNFα between ponies and horses.

Conclusion: The significant increase of SAA due to LPS challenge confirmed the procedure as model for systemic inflammation. This study demonstrated that non-obese ponies and horses did not show statistically significant differences in hepatic cytokine mRNA expression in a state of moderate systemic inflammation.

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



Median (whiskers indicate minimum and maximum) expression of CD68, chemerin, FABP1, IL1β, IL-6, LPL, progranulin and TNFα 15hours after LPS infusion in ponies and horses

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147.

Effects of glyphosate residues in animal feed on performance, energy metabolism and health characteristics in lactating dairy cows.

Einfluss von Glyphosatrückständen in Futtermitteln auf Leistung, Energiestoffwechsel und Gesundheit von laktierenden Milchkühen

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Glyphosate is worldwide the most used nonselective herbicide in agriculture; it inhibits the aromatic amino acid biosynthesis in plants. However, it is controversially discussed if it influences ruminal microflora and therefore the general performance and health of dairy cows. The aim of this study was to examine the influence of glyphosate residues in feedstuffs on performance, energy balance and health-related characteristics of lactating dairy cows.

Methods: 61 German Holstein cows (207 ± 49 Days in milk; mean ± SD) were used in a 17 week trial. At week 0, they were fed a ration consisting of 30% maize silage, 30% grass silage, 40% concentrate on a dry matter (DM) basis. In the next 16 weeks they were assigned to either a group receiving a glyphosate contaminated total mixed ration (TMR) (Gly) or a group receiving an uncontaminated TMR (Con). Straw and concentrate components originated from wheat and peas which, during growth on the field, were either untreated or treated with glyphosate following registered amounts. Each group was subdivided into a "low concentrate" group (LC) fed a diet composed of 21% maize silage, 42% grass silage, 7% straw and 30 % concentrate (on a DM basis) and a "high concentrate" group (HC) composed of 11% maize silage, 22% grass silage, 7% straw and 60% concentrate on DM basis for *ad libitum* consumption. Body condition score (BCS), body weight (BW) and dry matter intake (DMI) were recorded. At week 0, 7 and 15, general health status (0% = no symptoms, 100% = symptoms for all parameters) was evaluated by a modified clinical score according to Dirksen et al 2012 (1). Performance, energy metabolism and general health status data were analyzed using MIXED procedure of SAS Enterprise Guide 6.1.; values represent the Least Square-means ± Standard Error.

Results: Glyphosate (Gly) contamination did not affect BCS, DMI, net energy intake, net energy balance nor milk yield, whereas concentrate (C) and time (t) showed distinct effects for all parameters. There was an interaction between C and t; only milk yield showed an interaction between gly, C and t (Table 1). The health-score differed significantly over t and there was an interaction between gly, C and t. In the particular groups, the average glyphosate intake was Con LC 0.60 mg/d, Con HC 0.57 mg/d, Gly LC 62.18 mg/d and Gly HC 62.24 mg/d.

Conclusion: In the present study, glyphosate contaminated feedstuffs in the ration showed no influence on performance and energy balance of lactating dairy cows. The clinical examination showed no adverse effect of glyphosate contaminated feedstuffs on the cows´ health condition

Tab.1. Effect of glyphosate residues (gly), concentrate (C) and time (t) on performance and energy metabolism.

	Control (Con)		Glyphosate (Gly)		*p* -Value[3]		
	LC[1]	HC[2]	LC	HC	Gly	C	Gly*C*t
BCS	2.81 ± 0.11	3.15 ± 0.11	2.78 ± 0.12	3.01 ± 0.12	0.469	0.018	0.196
DMI, kg kg/d*	17.3 ± 0.3	19.7 ± 0.3	18 ± 0.3	19.4 ± 0.3	0.434	< 0.001	0.103
Net energy intake, Mk NEL/d	113 ± 2.4	140.8 ± 2.4	117.8 ± 2.6	141.2 ± 2.5	0.990	< 0.001	0.179
Net energy balance, MJ NEL/d	3.9 ± 2.2	32.7 ± 2.2	7.5 ± 2.4	32.6 ± 2.3	0.769	< 0.001	0.819
Milk yield, kg/d	21.2 ± 1.0	26.7 ± 1.0	21.1 ± 1.1	26.7 ± 1.0	0.933	< 0.001	0.011
Clinical health score, %	5.15 ± 0.44	4.53 ± 0.45	4.87 ± 0.47	4.9 ± 0.46	0.926	0.525	0.010

[1]Low concentrate ration [2]High concentrate ration [3]gly*C, gly*t ($p > 0.05$) for all variables; t ($p < 0.001$) for all variables except BCS ($p = 0.021$), C*t ($p < 0.001$) for all variables except clinical health score ($p = 0.276$).

1) DIRKSEN, et al.,-2012- ENKE, STUTTGART

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148.

Effects of an intramammary lipopolysaccharide challenge on metabolic responses and liver enzymes in dairy cows experiencing or not subacute ruminal acidosis

Einfluss einer intramammären Lipopolysaccharid-Challenge auf Stoffwechselmetabolite und Leberenzme bei Milchkühen mit oder ohne subakuter Pansenazidose

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Feeding high grain diets increases the risk of subacute ruminal acidosis (SARA) and might modulate the responsiveness to external immunogenic compounds such as bacterial lipopolysaccharide (LPS). We recently observed a more pronounced depression in feed intake, chewing behavior, and milk production in cows experiencing SARA compared to healthy cows after receiving an intramammary LPS infusion (1). This study investigated the stress, metabolic health, calcium (Ca) and liver health responses of dairy cows submitted to an experimental LPS-induced mastitis challenge and experiencing SARA conditions or not. **Methods:** Eighteen early-lactating cows were randomly assigned to two different feeding regimens, control (n = 6) and SARA (n = 12). The control cows (CON) received a diet containing 40% concentrates throughout the experiment. The SARA cows were subjected to an intermittent feeding regimen, consisting of a diet with 60% concentrates for 32 d with a break with feeding 40% concentrates for 7 d in-between. On d 30 of the experiment, a single dosage of 50 µg LPS from E. coli (O26:B6) was intramammarily administrated into the left front quarter of all CON cows (CON-LPS) and half of the SARA cows (SARA-LPS). The remaining six SARA cows (SARA-PLA) received a sterile NaCl solution as placebo. To optimally mimic natural E. coli mastitis LPS was administrated intramammary. Blood samples were collected at several time points during the 30 d feeding trial as well as shortly before and at 6, 12, 24, and 48 h after the LPS/PLA infusion. Blood samples were analyzed for cortisol, Ca and several metabolites related to glucose and lipid metabolism as well as for reactants of liver health such as aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and glutamate dehydrogenase (GLDH). Data were analyzed using the MIXED procedure of SAS. **Results:** Prior to LPS/PLA infusion, the SARA-cows showed higher blood glucose (P=0.02), a tendency for greater lactate (P=0.07) and lower GGT (P=0.08) as well as beta-hydroxybutyrate (BHBA; P=0.09) compared to CON cows. After the intramammary LPS infusion, the cortisol concentration markedly increased (P **Conclusions:** Data suggest that a single intramammary LPS infusion increased the cortisol level and lowered Ca concentration in all challenged dairy cows, whereby the latter has been suggested as mechanism to facilitate the detoxification of LPS (2). Overall, a hypoketonic and hyperlactemic effect was only evident in cows experiencing intermittent SARA conditions.

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2) Eckel, F.E., and B.N. Ametaj. 2016. J. Dairy Sci. 99:5967-5990.

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149.

Measurement of abomasal luminal pressure, pH and temperature in healthy and diarrheic dairy calves using a wireless ambulatory capsule (SmartPill®)

Luminaler Druck, pH und Temperatur im Labmagen bei gesunden und durchfallkranken Kälbern unter Einsatz eines telemetrischen Messsystems (SmartPill®)

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Neonatal diarrhea is the main cause of death during the first two weeks of life in calves. Furthermore diarrhea results in poor growth performance and in a higher susceptibility towards other infections. As recently described by Kirchner et al. (2015) neonatal diarrhea has an impact on abomasal motility, in consequence abomasal emptying rate is delayed after milk intake. Impaired abomasal motility may increase bacterial fermentation and the production of short chain fatty acids. The aim of the study was to investigate abomasal luminal parameters in healthy and diarrheic calves by a wireless ambulatory capsule (WAC). Additionally, acetaminophen absorption test (APAT) was used to determine abomasal emptying rate.

Methods: Nine female Holstein-Frisian calves (age <14 days, BW ± SD: 43.7 ± 6.3 kg, 4 healthy calves (H) and 5 acute diarrheic calves (D, fecal score ≥ 2 on a scale of 0 to 3)) were included in the study. For APAT calves were fed exclusively 2 L of milk replacer containing 50 mg acetaminophen/kg body weight after an overnight fast. Blood samples were taken for the analysis of acetaminophen (AAP) over a time period of 12 hours after milk replacer intake. Concomitant, a WAC was orally applied into the abomasum for the continuous measurements of luminal pH, pressure and temperature.

Data analysis was performed by statistical software programs (Statistica 7.1, StatSoft). Normally distributed data were subjected to unpaired t-test. In case of not normally distributed data, data were subjected to Mann-Whitney U test. Statistical significance was accepted at $P<0.05$. A trend was postulated at $P<0.1$.

Results: Calves with diarrhea tested positive for Rota virus and Cryptosporidium parvum. Three of the five diarrheic calves were infected with Corona virus, and Escherichia coli was found in two of five fecal samples. Immediately after feeding mean abomasal pH varied around 5.77 ± 0.69 with a minimum of 4.58 and a maximum of 6.60. Postprandial, the intraluminal pH decreased until a pH<2 in both groups. The mean time of reaching a pH<2 was 338 ± 59.3 min (range: 255 - 426 min) after feed intake. 360 minutes after feeding pH varied around 1.95 ± 0.77 for H and 1.89 ± 0.63 for D. After feed intake mean abomasal temperature increased continuously until 39.0 ± 0.3°C at t=240 min in H and 39.6 ± 0.4°C at t=540 min in D, respectively. Abomasal temperatures of healthy and diarrheic calves were similar in the first 420 min postprandial. Within the last five hours of observation (t= 420 to 720 min post prandial) significantly higher temperatures were measured in D than in H. The mean maximum observed AAP concentration of D (45 ± 12 µg/mL, range from 29 to 51.5 µg/mL) was not different from mean maximum AAP concentration of H (59.5 ± 8 µg/mL, range from 50.7 to 66.4 µg/mL, P=0.13). The AAP half-time was more than one hour faster in H ($t1/2$ =171 ± 36 min) than in D ($t1/2$ =245 ± 42 min, P = 0.04).

Conclusion: Abomasal environment was different between diarrheic and healthy calves. Significant differences in AAP half-time reflect a delay in abomasal emptying in diarrheic calves. An increased abomasal temperature may indicate bacterial fermentation processes during prolonged abomasal retention time which had to be considered in feeding management of diarrheic calves.

1) Kirchner, D., Schwedhelm, L., Wenge, J., Steinhöfel, I., Heinrich, C., Coenen, M., Bachmann, L. (2015): Ultrasonographic imaging of abomasal milk clotting and abomasal diameter in healthy and diarrheic calves. Animal Science Journal 86, 929-936.

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150.

Systemic availability of quercetin after oral application of quercetin or rutin in miniature Shetland ponies

Bioverfügbarkeit von Quercetin nach oraler Applikation von Quercetin oder Rutin bei Mini-Shetlandponys

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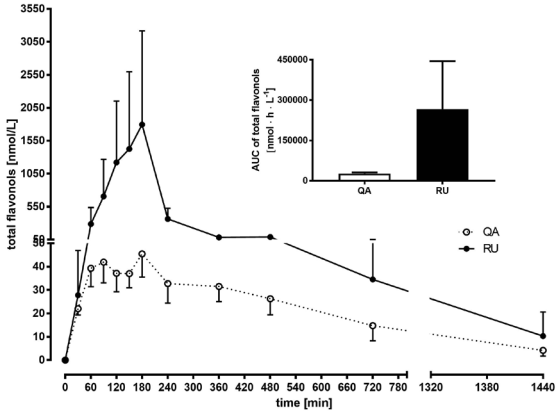
The flavonol quercetin shows anti-oxidative, anti-inflammatory and other health-promoting properties *in vitro* as well as *in vivo*. Previous studies on the bioavailability of quercetin in monogastric species (man, pig, rat, dog) consistently revealed, that ingestion of the quercetin aglycone (QA) resulted in higher quercetin plasma concentrations compared to the application of the quercetin glucorhamnosid rutin (RU). However, in cows the intraruminal application of RU resulted in a higher bioavailability of quercetin compared to QA. The aim of the present study was, to investigate the bioavailability of quercetin from RU and QA, respectively, in horses.

Methods: Four adult healthy miniature Shetland ponies with a mean body weight (BW) of 120 ± 11 kg were used. Animals were fed with sugar-free beet-pulp (70 g + 350 g water at 8 am) and hay (*ad lib*). During the pre-experimental (14 days) and experimental phase (8 days) pasture and intake of dietary supplements, apples as well as medication was excluded. During the experiment, QA and RU, respectively, (50 mg quercetin-equivalents/kg BW) were once given with the morning meal. Animals were allocated for a latin square design (two-sided, paired t-test) with a wash out period of 7 days. Ingestion of the test meal was completed within 10 min. One hour after completion of the test meal hay and water were offered for *ad libitum* intake. Blood samples (10 ml) were collected into heparinized blood containers from the jugular vein once directly before and frequently (Fig. 1) after intake of the test meal over a period of 24 h. Flavonols in plasma (quercetin, kaempferol, isorhamnetin) and feed were analyzed by HPLC with fluorescence detection.

Results: The maximum plasma concentration of total flavonols (sum of quercetin, kaempferol, and isorhamnetin) was reached 180 min after application with both quercetin sources. Maxium plasma concentration as well as the area under the plasma concentration time curve (AUC) were higher after oral application of RU compared to QA (paired t-test, p = 0.023 and 0.0625, respectively (Fig. 1).

Conclusions: From the present study we conclude, that in miniature Shetland ponies - similar as in cows - the systemic availability of quercetin is higher after application of R compared to QA. Higher plasma concentrations of total flavonols could be explained either by microbial liberation of quercetin from RU within the pars proventricularis of the stomach with subsequent absorption from the small intestine whereas quercetin from QA may be degraded more rapidly. Alternatively, liberation and absorption of quercetin from QA in the large intestine might also be involved.

Figure1: Plasma concentrations of total flavonols after oral application of quercetin aglycone (QA) or rutin (RU) (50 mg quercetin equivalents/kg BW); data are means ± SD (n = 4); * different between the application of QA and RU; paired t-test, significant at p = 0.023 *Inset:* Area under the concentration time curve (AUC) of total flavonols; * different between the application of QA and RU; paired t-test, strong trend at p = 0.0625



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151.

Pilot study about day-to-day variation of HCl-insoluble ash in faeces of horses

Orientierende Studie zur zeitlichen Variation der Gehalte an HCl-unlöslicher Asche im Pferdekot

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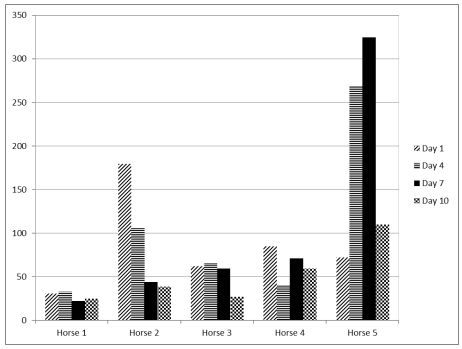
The content of HCl-insoluble crude ash is used in equine veterinary practice to estimate the load of the equine gastrointestinal tract (GIT) with sand and to monitor therapeutic measures. Intake of high amounts of sand is associated with a higher risk of colic (1) and therefore this should be avoided. It is well known that horses kept on sandy paddocks or "poor pasture" are of higher risk to show a higher sand intake. Although rough critical border lines can be found in literature - values below 100 g/kg dry matter of faeces are assumed to be not critical (2) - information about the variation within time is scarce. This study aimed to determine the content of HCl-insoluble ash during course of time in faeces of horses kept under constant conditions, to test whether a single sample is suitable to evaluate the sand load.

Material and Methods: 5 healthy horses owned by the equine clinic Lüsche since several years were used for this orientating study. The horses (mean age 12.8 ± 4.15) were housed in individual boxes during night and had access to pasture (grass height: ~ 10 cm) for about 5 hours per day (except for horse 2 which was housed on a sand paddock). The horses were fed a constant diet based on hay (12kg) and oats as well as a pelleted mixed (3kg) feed in three meals per day. Fresh faeces of these 5 horses were taken on day 1, 4, 7 and 10 in the morning (between 7am and 8am) to determine the day-to-day variation of HCl-insoluble crude ash content. The faeces were dried, ground and crude ash as well as HCl-insoluble ash were determined by established methods (3)

Results: Most values of HCl-insoluble ash were below 100 g / kg dry matter - with only two horses showing values above 100 g / kg dry matter. The day-to-day individual variation of HCl-insoluble ash differed markedly between horses. While in some horses the variation was very low, there was a variation of 72 g/kg dry matter (day 1) up to 325 g/kg dry matter (day 7) in horse 5 (see figure 1).

Figure 1: Content of HCl-insoluble ash in faeces (g/kg dry matter) of horses (day 1, 4, 7 and 10)



Discussion: The high day-to-day variation of sand in the faeces of horses kept under constant conditions is surprising and raises the question, whether the analysis performed routinely to detect increased sand load in the GIT of horses (spot sampling on one day) is suitable. Taking into account the borderline of 100 g HCl-insoluble ash / kg dry matter the elevated values of horse 2 and 5 would have been detected only on days 1 and 4 resp. 4 and 7. It is known that the critical value of sand within the GIT - causing clinical symptoms - differs widely on individual basis (4). The finding that faecal excretion differs markedly from day to day under constant feeding and housing conditions underlines the high complexity of the diagnosis "sand load" of GIT of horses in practice.

Conclusion: The HCl-insoluble ash content in equine faeces showed a high day-to-day variation. Therefore repeated measurements are needed to gain profound information about the sand load of individual equine GIT. Further studies will be performed to investigate the variation of faecal sand excretion more into detail to be able to give recommendations for suitable test procedure.

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152.

Different inflammatory cytokine expression in several adipose tissue localisations in non-obese ponies and horses undergoing a lipopolysaccharide (LPS) challenge

Unterschiedliche inflammatorische Zytokinexpression in verschiedenen Fettdepots bei normalgewichtigen Ponys und Pferden nach einer Lipopolysaccharid-Provokation

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Ponies are more prone to suffer from metabolic diseases compared to horses. However, it is still under discussion why ponies are more susceptible to laminitis and equine metabolic syndrome (EMS). Research suggests another trigger (e.g. LPS) to induce metabolic laminitis besides obesity. A LPS challenge is an established and well repeatable method to induce a mild systemic inflammatory state. It is speculated that different adipose tissue (AT) localisations (for example subcutaneous (sc) AT over the nuchal ligament) are metabolic more active with respect to inflammatory cytokines than others. This study is part of a larger project investigating the different cytokine expressions of ponies and horses undergoing a LPS challenge with increasing obesity. In this particular part of the study we hypothesize that lean ponies react with a higher mRNA expression of inflammatory cytokines undergoing a LPS challenge compared to lean horses.

Methods: 10 lean adult ponies and 10 lean adult horses: (median (25./75. percentile) body condition score: ponies = 3.65 (2.15/4.4); horses = 3.57 (3.05/4.2) on a scale out of 6; insulin sensitive: median (25./75. Percentile) basal serum insulin 5.27µU/mL (4.18/5.93µU/mL) underwent an intravenous LPS challenge with 10 ng/kg BW LPS (O55:B5 Escherichia coli). 15 hours after LPS challenge AT of four localisations (retroperitoneal of the margins of the abdominal incision, visceral of the mesocolon of the colon descendens, sc tissue of the nuchal crest and lateral the tail head) was collected under general anesthesia. The mRNA expression of genes of interest (chemerin, CD68, IL-1β, IL-6, progranulin and TNFα) and of reference genes (18S and RPL32) were analysed using RT-qPCR. The genes of interest were normalized against a geometrical mean of the reference genes. Retroperitoneal AT and visceral AT were pooled as abdominal AT. The two sc AT localisations were also pooled. Mann-Whitney-U test was performed to compare mRNA expression of ponies and horses and to compare mRNA expression of abdominal AT and subcutaneous AT. Significance was accepted at $P < 0.05$.

Results: The mRNA expression of the inflammatory cytokines IL-1β, IL-6 and TNFα was above the expression of the reference genes in all localisations. The expression of the other genes of interest was very low compared to the reference genes. For significant different mRNA expression between ponies and horses see table.

Table: m-RNA expression (x-fold) of selected parameters in the subcutaneous AT of the tail head comparing ponies with horses

Parameter	Localisation	Breed	n	median	25./75. percentile	P-value
IL-1β	tail head	ponies	10	3.423	3.035/1.748	0.017
	tail head	horses	10	2.247	1.748/2.809	
IL-6	tail head	ponies	10	3.820	3.093/4.948	0.009
	tail head	horses	10	2.386	1.876/2.788	

In ponies the expression of IL-6 was significant higher in the sc AT compared to the abdominal AT ($P = 0.01$).

Conclusion: The ponies had a significant higher expression of the typical inflammatory cytokines like IL-1β and IL-6. But we observed this difference only in the sc AT of the tail head. We did not observe clear differences in mRNA expression in each localisation between ponies and horses. However, we expect stronger differences between horses and ponies during progressive weight gaining.

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

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153.

Field Study to investigate the informative value of allergene screening tests based on IgE ELISA in horses with respiratory symptoms or skin diseases

Feldstudie: Untersuchungen zur Einschätzung der Aussagekraft von Ergebnissen eines ELISA basierten IgE Screening-Allergietest bei Pferden mit Atemwegs- oder Hauterkrankungen

*Penning M., Swagemakers J.-H., Mößeler A., Kamphues J. – Lüsche/Hanover

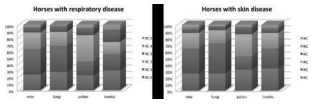
In equine clinical veterinary practice it is on debate, whether the prevalence of allergies in equine patients has increased in recent years and whether the tests used routinely are suitable to detect relevant allergic reactions. In equine practice it is often suspected that skin diseases (supposed reaction on insects), as well as respiratory disturbances (supposed main allergens: mites and fungi) are related to allergic reactions. An accurate diagnosis is essential for therapy - nonetheless the value of the commonly used allergy tests (based on ELISA) is discussed controversially. This study aimed to evaluate retrospectively the results of "allergy tests" in equine patients with respiratory symptoms and skin affections at the equine clinic in Lüsche, Germany, from 2013 to April 2016.

Material and Methods: Results of allergy tests (screening) of 211 patients (regular curative activities) with symptoms suspected to be related to allergic reactions were evaluated. Blood was obtained by venipuncture (Vena jugularis), centrifuged and serum was sent to a commercial external laboratory (Laboklin, Bad Kissingen, Germany). The level of IgE in serum samples was analysed by ELISA and results were classified by the laboratory into 6 reaction classes (RC) from RC 0 (no reaction) to RC 5 (highest intensity of reaction) according to the intensity of IgE response. The four main allergens investigated on a regular basis by the lab were mites, fungi, pollen and insects.

Results: At least 69 % of the horses were classified in RC 1 or higher with most horses showing only a mild reaction (RC 1 or 2). Only few horses were classified with RC 4 or 5. Many horses showed positive reactions to more than one of the four allergen groups. In 95 of the 211 horses the allergy test was performed by virtue of respiratory disease. Most of these horses showed a mild reaction (RC 1 or 2; see table) on mites, fungi and pollen. Also most horses with skin disease were classified at RC 1 or 2 (see figure). There were no significant differences in the distribution of the reaction classes between horses with respiratory diseases and those with skin symptoms.

Figure 1: Relative distribution of RC in horses with respiratory symptoms (Resp.) or skin diseases

Distribution	Mite		Fungi		Pollen		Insects	
of RC %	Resp.	Skin	Resp.	Skin	Resp.	Skin	Resp.	Skin
RC 0	23.96	26.00	28.13	26.00	22.92	32.00	20.21	34.00
RC 1	38.54	38.00	39.58	46.00	20.83	20.00	25.00	30.00
RC 2	26.04	24.00	21.88	20.00	40.63	34.00	18.75	24.00
RC 3	7.29	6.00	8.33	6.00	10.42	10.00	17.71	6.00
RC 4	3.13	2.00	2.08	0.00	2.08	2.00	6.25	6.00
RC 5	1.04	4.00	0.00	2.00	3.13	2.00	2.08	0.00



Discussion: As the horses tested were patients with signs suspected to be related to allergic reactions, these data are not representative for the whole population (negative selection). The use of IgE response in horses for diagnosis is under critical debate as "non allergic, clinically healthy horses" often also develop allergen-specific IgE antibodies" (1) and results of the commercially available IgE based tests on feed allergens were inconsistent in healthy ponies (2). Unexpectedly, the results of horses with skin and respiratory symptoms did not differ. The supposed higher reaction of horses with respiratory symptoms regarding mites and fungi was not confirmed.

Conclusion: Although these tests are currently widely used in clinical practice the diagnostic value seems to be limited as the prevalence of free IgE is not necessarily a suitable marker to prove clinical relevance (1, 2). The finding that RC of horses suffering from diseases of the respiratory tract and skin did not differ according to the different allergens is surprising as this finding is in contrast to the daily practice and draw further critic on this test procedure.

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154.

Relationships between feed intake, chewing activity, diet digestibility, and rumen microbial protein synthesis in lactating dairy cows

Korrelationen zwischen der Futteraufnahme, Kauaktivität, Verdaulichkeit und ruminalen mikrobiellen Proteinsynthese bei laktierenden Milchkühen

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Feed intake, diet digestibility, and rumen microbial protein synthesis greatly determine energy and amino acid supply and thus productive performance in dairy cows. Chewing activities may enhance nutrient degradability and microbial growth in the rumen by reducing feed particle size and improving access of microbes to intracellular nutrients. However, the impact of feeding and rumination activities of individual animals on diet digestibility and rumen microbial protein synthesis has not yet been unraveled. Hence, this study investigated the interrelationships between feed intake, diet digestibility, chewing activity, and rumen microbial growth in lactating dairy cows.

Methods: Eighteen lactating Holstein-Friesian cows (mean bodyweight 673 kg (standard deviation 69) and mean milk yield 34 kg/d (standard deviation 8)) were monitored during two periods of 10 d each. Their diet consisted of maize silage, grass silage, grass hay aftermath, barley straw, and a concentrate mixture offered as total mixed ration. Samples of feed, milk, and excreta were collected on days 6-10. Apparent total tract organic matter digestibility (OMD) was estimated from crude protein concentrations in fecal organic matter by an exponential regression model (1). Concentrations of purine derivatives (PD) and creatinine in urine were used to calculate the PD to creatinine (PDC) ratio and the PD to creatinine index (PDCI) as indicators for rumen microbial protein synthesis (2). Individual chewing behavior was recorded with noseband pressure sensors (RumiWatch, Itin & Hoch). Correlation analysis was conducted with SAS (V9.4).

Results: Intakes of dry matter ($r = -0.49$, $p = 0.041$) and neutral detergent fiber ($r = -0.55$, $p = 0.017$) were negatively correlated with OMD (Table 1). The fat- and energy-corrected milk (FECM) yield increased with increasing dry matter intake and was also positively correlated to indicators of rumen microbial protein yield. Rumination time positively correlated with PDC, PDCI, and FECM. While rumination time correlated negatively with OMD, the latter increased with increasing rumination velocity (chews/min; $r = 0.64$, $p = 0.006$). Rumination chews per kg of dry matter intake did not influence OMD, but were negatively related to PDCI ($r = -0.46$, $p = 0.060$).

Table 1 Pearson correlation coefficients (r) and probability (p) of correlations between feed intake, milk yield, diet digestibility, chewing activities, and indicators of rumen microbial protein synthesis as determined in lactating dairy cows (n = 18).

Variable	Unit		DMI	FECM	PDCI	PDC	RT	RC
DMI	kg/d	r	1					
		p						
FECM	kg/d	r	0.69	1				
		p	0.001					
PDCI		r	0.81	0.78	1			
		p	<0.001	<0.001				
PDC		r	0.53	0.77	0.89	1		
		p	0.023	< 0.001	< 0.001			
RT	min/d	r	a	a	0.43	0.60	1	
		p			0.086	0.011		
RC	n/d	r	a	a	a	0.42	0.82	1
		p				0.095	< 0.001	
OMD	g/kg OM	r	-0.48	a	-0.69	-0.71	-0.43	a
		p	0.041		0.002	0.001	0.045	

'a' p-values>0.1. DMI, dry matter intake; FECM, fat- and energy-corrected milk; PDCI, purine derivative to creatinine index; PDC, purine derivative to creatinine ratio; RT, rumination time; RC, rumination chews; OMD, apparent total tract organic matter digestibility; OM, organic matter.

Conclusions: The results support the strong interrelationships between feed intake, diet digestibility, and rumen microbial growth, but also suggest that differences in the chewing behavior of individual animals may influence OMD and duodenal microbial protein flow in one the same diet. Findings should however, be confirmed for other dairy cattle diets.

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155.

Spatial heterogeneity of vegetation and grazing behavior of cattle on pastures in the Peruvian Andes

Räumliche Heterogenität in der Vegetation und dem Weideverhalten von Milchkühen in den Anden Perus

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Under grazing conditions, selective patch grazing may reduce overall forage use efficiency and productivity of ruminant livestock (1). It may enhance plant regrowth and thus nutritional value of forage at preferred foraging sites which in turn could increase nutrient and energy intakes of animals, for instance, in grasslands characterized by low-quality forage (2). Understanding the spatial heterogeneity of the vegetation of grasslands and their utilization by grazing animals can thus contribute to enhance grazing management and herd performances. The objective was therefore to characterize the spatial distribution of plant biomass and its nutritional composition and to analyze its relation to the behavior of grazing cows on natural pastures of the Central Andes of Peru.

Methods: The study was conducted in the Chalhuas community (S11 57 924, W75 32 995; 3.860 m above sea level), Central Andes of Peru, during February - May 2015. On pastures (20 - 26 ha) of three farms, geographical position changes of five lactating Criollo x Brown Swiss cows each were recorded every 30 sec for 10 - 12 d per pasture using global positioning system recorders. Their *grazing* behavior was classified from positioning data using region-specific velocity thresholds. Above-ground plant biomass was determined along transects by destructive sampling in 1-m²-plots (n = 27 - 44 per farm) that were evenly distributed across the pastures. Individual forage samples were analyzed for dry matter (DM), crude protein, and neutral detergent fiber. *Kernel density distribution was estimated for foraging, resting, and walking without foraging using ArcMap 10.3 (Esri, Redlands, USA). Correlation analyses were conducted between vegetation parameters and the probabilities of different behavioral activities at individual sampling plots of all pastures using R software 3.2 (R-Studio, Boston, USA).*

Results: The above-ground plant biomass ranged between 216 - 857 kg DM/ha with concentrations of crude protein and neutral detergent fiber of 47 - 192 g/kg DM and 515 - 770 g/kg DM, respectively. On average (± standard deviation), the animals spent 9.8 ± 0.10 h/d foraging and 2.0 ± 0.09 h/d walking during which they covered distances of up to 7.8 km/d. Due to the fact that cows were kept in paddocks around milking and parts of the night, they spent 12.3 ± 0.09 h/d resting and for other activities. Both, plant biomass and its crude protein concentration varied greatly across the pastures of the three farms with coefficients of variations of 0.16 - 0.29 and 0.16 - 0.33, respectively. Similarly, areas of the pastures that were preferably grazed or rejected by cows could be identified. Nevertheless, there were no significant correlations between individual vegetation parameters and the probability of foraging across all sampling points and pastures *($P > 0.05$)*.

Conclusions: Mass and nutritional quality of herbaceous vegetation available on pastures during the rainy season covers nutrient and energy requirements of dairy cows for maintenance, activity, and a milk yield of 3.1 - 3.2 kg/d. Selection of grazing sites appears to be not only determined by mass and nutritional value of the available forage in different areas of the pastures. Pronounced spatial differences in the grassland vegetation and an uneven distribution of the impacts of animal grazing should be considered when optimizing grassland use for milk production in the Peruvian Andes.

This study was financially supported by the Fiat Panis foundation.

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156.

Plasma thyroid hormone concentrations in dairy cows of high versus normal body condition

Plasma-Schilddrüsenhormon-Konzentrationen in über- und normalkonditionierten Milchkühen

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Thyroid hormones, together with other hormones, play a pivotal role in mammary gland development and function, and are thus important for milk production. Moreover, thyroid hormones are recognized as the major endocrine regulators of metabolic rate. Thyroxine (T_4), which is considered a prohormone, and the metabolically active triiodothyronine (T_3) are both knowingly decreased during early lactation, leading to a physiological hypothyroid state (1). Over-conditioned cows bear a greater risk for developing metabolic diseases when entering lactation due to excessive lipolysis and insufficient fatty acid oxidation. In view of recent reports about a linkage between thyroid hormone signaling and specific mitochondrial-targeted pathways (2), as well as the discovered associations between thyroid status and body fat portion in humans (3), we aimed to compare dairy cows of different body condition during the transition from pregnancy to lactation with regard to their plasma concentration of T_3 and T_4.

Methods: 15 wk before calving, 33 pluriparous Holstein Friesian cows were allocated based on their previous course of body condition score (BCS) and back fat thickness (BFT) to either a normal-conditioned (NBCS) group or an over-conditioned (HBCS) group. To further amplify BCS and BFT differences between the groups, the NBCS cows received an energy-reduced ration [6.6 MJ NEL/kg dry matter (DM)], whereas the HBCS cows received a high-energy ration (7.1 MJ NEL/kg DM) from wk 15 ante partum until dry-off (wk -7). During the dry period and the subsequent lactation both groups were fed the same diet. Plasma free T_3 (fT_3) and T_4 (fT_4) concentrations were measured on d -49, -21, -7, 3, 14, 21, 35, 49, 63 and 84 relative to calving by immunoassay (Dimension Vista; Siemens Healthcare Diagnostics, Eschborn, Germany). The assay was validated for bovine samples. The obtained data were analyzed by Linear Mixed Model and Bonferroni post-hoc tests. Spearman's correlation coefficients were calculated to test for relations between different variables. The level of significance was set at $P \leq 0.05$.

Results: The preselection of the cows and the differential feeding before dry-off led to differences in body condition ($P < 0.05$): BCS and BFT values around calving were 3.44 ± 0.11 and 1.30 ± 0.08 cm for NBCS versus 4.2 ± 0.06 and 2.32 ± 0.09 cm for HBCS cows, respectively. The concentrations of fT_3 and fT_4 changed with time in both groups ($P < 0.05$), i.e. from d - 21 until d 3, concentrations dropped approx. by 17% for fT_3 and by 38% for fT_4. Thereafter the same levels were maintained until the end of trial. There was no group difference for fT_3, but treatment by time interactions were observed for fT_4: HBCS cows had greater fT_4 concentrations (1.09 ± 0.05 ng/dL) than NBCS cows on d -49 ($P < 0.05$); the group difference became smaller on d -21 ($P < 0.1$) and disappeared thereafter. The fT_3: fT_4 ratio increased with time in both groups. Considering all time points and animals, weak positive correlations ($P \leq 0.001$) between fT_3 and BCS ($r = 0.375$) and BFT ($r = 0.208$) were found. Similarly, fT_4 and BCS ($r = 0.376$) and BFT ($r = 0.274$) were correlated ($P < 0.001$), whereas no associations were found between the fT_3: fT_4 ratio and BCS or BFT.

Conclusions: With the exception of fT_4 for which initial differences were detectable at dry-off, cows with different BCS hardly differed in their plasma concentration of fT_3 and fT_4, and the ratio calculated therefrom. The greater fT_4 concentrations in HBCS cows observed at dry-off may result from systemic effects triggered by the preceding differential feeding and/or from differences in adipose tissue thyroid hormone metabolism. However, the following switch to a common feeding regimen as well as parturition and lactation associated changes may have overridden the initial differences.

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157.

Effects of a ration change from a silage and concentrate- to a pasture-based diet on the motoric activity and rumination of dairy cows

Einfluss eines Rationswechsels von einer totalen Mischration auf eine weidebasierte Fütterung auf das Aktivitäts- und Wiederkauverhalten von Milchkühen

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The transition from a total mixed ration (TMR) to pasture in spring requires metabolic and behavioral adaptations. The aim of this study was to examine the changes in animal behavior upon this nutritional change using continuous eating behavior measuring devices and pedometers. The study is part of a research project of Lower Saxony called: "The production of milk in pasture- or confinement-based systems".

Methods: Eleven German Holstein cows (26.9 ± 6.1 kg milk/d, 156 ± 31 d in milk, means ± SD) were divided into a pasture- and confinement group (PG, n=6; CG, n=5). The CG received a TMR (35% maize silage, 35% grass silage, 30% concentrate, DM-basis) throughout the trial, whereas the PG was gradually transitioned to pasture (wk0 and 1: TMR-only, wk2: 3 h/day on pasture, wk3 and 4: 12 h/day on pasture, wk5 to 11: pasture-only plus 4.35 kg concentrate/d). The cows were alternately equipped with a sensor-based automatic measurement system (RumiWatch, Liestal, Switzerland; during 3 ± 1 d per wk, means ± SD) to record the time per day the animals were ruminating (RT), eating (ET), standing, laying or walking (WT). Measurements were analyzed using PROC MIXED in SAS Enterprise Guide 7.1.

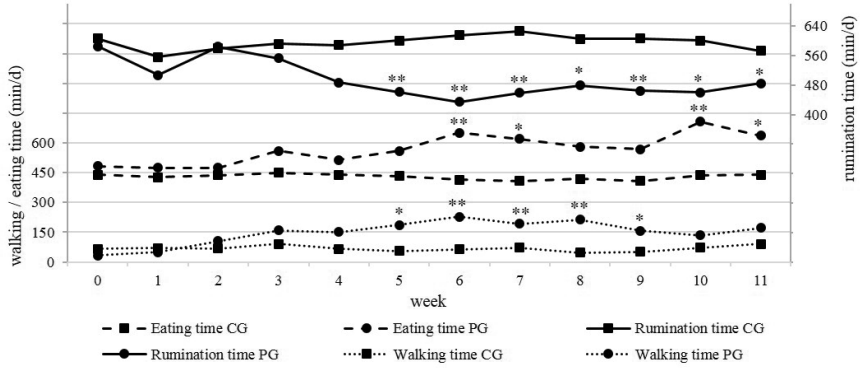

Figure 1. Effect of a ration change from TMR to pasture on walking time, eating time and rumination time. LSMeans. Significances: WT: Group (G): P < 0.01, Week (Wk): P < 0.05, G*Wk: P < 0.05, PSEM: 17; ET: G: P < 0.01, Wk: P < 0.01, G*Wk: P < 0.01, PSEM: 34; RT: G: P < 0.01, Wk: P = 0.21, G*Wk: P < 0.05, PSEM 30; symbols indicate significant differences between groups in particular week: * P ≤ 0.05; ** P ≤ 0.01; MIXED procedure in SAS Enterprise Guide 7.1 (SAS Inc. 2014, Cary, NC, USA).

Results: The WT increased continuously in the PG until wk6 and decreased again thereafter (Figure 1). The ET increased until wk6, decreased thereafter until wk9 and increased again until wk11. The RT decreased in the PG from wk2 until wk6 and exhibited a slight increase until the end of the trial. No significant Group or Group*Week effects were observed for the other behavior variables.

Conclusion: Present results show that the behavioral adaptation required several weeks. Further analysis of feed composition/production, metabolic and rumen fermentation data is needed to prove whether the decreased rumination time can be attributed to an increased time required for grazing or to a different fiber content of the diet.

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158.

Chemical body composition of individual newborn piglets with different weights at birth

Zur Körperzusammensetzung neugeborener Ferkel bei unterschiedlichem Geburtsgewicht

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Productivity of modern high reproductive sows steadily increased in recent years. This led to an average of 28.5 weaned piglets per sow and year [1]. But increasing litter size is correlated with lower birthweights and enlarged variation within litters and therefore the percentage of piglets born with less than 1.0 kg bodyweight increased from 3% to 15% [2]. Compared to former times the ability of underweight piglets to survive seems to be increased, and the definition of "underweight" piglets is on debate. The hypothesis of this study was that nowadays the piglets with lower birth weight differ in their composition in comparison to former times.

Material and methods: 12 newborn piglets (both sexes, genetic line: BHZP), either stillborn, born alive or squashed by the sow within the first 24 hours of life, were allotted to three groups (n=4) depending on their body weight at birth (less than 0.8 kg BW; 0.8-1.2 kg BW; >1.2-1.6 kg BW). The piglets were stored frozen at -21□, shred in a common meat cutter, freeze-dried and ground in the Grindomix GM 200 (Fa. Retsch, Haan, Germany). An aliquot was taken and ground again in the Mixer Mill MM 400 (Fa. Retsch, Haan, Germany) to reach a high level of fineness (approximately 5µm) and homogeneity. A representative sample out of the finely ground material was analysed by Weende analysis with exception of the crude fibre content. Statistical analyses were performed by using the SAS® software (Cary, NC, USA), using the t-test for normally distributed data and Wilcoxon-test (not-normally distributed data). $P < 0.05$ was considered to be statistically significant.

Results: The chemical composition of underweight piglets with less than 0.8 kg weight at birth differed markedly from the composition of piglets with "normal" birth weight. The content of carbohydrates showed main differences and furthermore the crude fat content influencing also the gross energy (GE) content. In contrast to the carbohydrates and crude fat contents the crude protein contents of underweight piglets were increased compared to former times [3].

Tab. 1: Body composition and energy content in newborn piglets of different BW classes

Piglet's BW	(kg)	<0.8	0.8-1.2	>1.2-1.6
DM	(g/kg)	$192^{ab} \pm 17.3$	$202^{a} \pm 6.11$	$192^{b} \pm 5.72$
Crude ash	(g/kg DM)	$236^{a} \pm 28.0$	$187^{a} \pm 5.36$	$195^{a} \pm 13.2$
Crude protein	(g/kg DM)	$645^{a} \pm 45.6$	$622^{a} \pm 12.6$	$603^{a} \pm 15.0$
Crude fat	(g/kg DM)	$58.4^{b} \pm 3.40$	$77.3^{a} \pm 8.34$	$66.1^{ab} \pm 6.36$
Carbohydrates[1]	(g/kg DM)	$61.3^{b} \pm 34.8$	$114^{a} \pm 16.1$	$136^{a} \pm 28.7$
GE	(MJ/kg DM)	$18.8^{b} \pm 0.709$	$20.0^{a} \pm 0.311$	$19.4^{b} \pm 0.216$

[1] mainly glycogen; calculated: DM-(ash, protein, fat)

[a-b] different superscripts within a row indicate significant differences ($p < 0.05$)

Except calcium and phosphorus (higher contents in lightweight piglets), the macro mineral contents on a DM-basis were similar in the different body weight classes. Regarding the trace element contents lightweight newborn piglets seemed to have higher zinc contents.

Conclusion: The data set has to be completed before it can be used for further calculations on pregnant sows´ nutrient requirements. In contrast to 1975 newborn piglets <1.2 kg weight at birth can´t be classified as underweight ones. That can be confirmed by the results of Weende analysis. It has to be discussed which factor is decisive whether a newborn piglet with 0.8 kg BW is equipped sufficiently to survive. Confirmed by a higher number of samples, the carbohydrate reserves might be the limiting factor but this presumption has to be regarded critically because it can´t be denied that some of the piglets had an intake of colostrum before. Under the condition of today`s high reproductive performance the classification of 1975 [3] is not recommendable anymore because a "normal" body composition was found also in newborn piglets with birthweight of about 1 kg.

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159.

Effect of formula-feeding on the susceptibility of neonatal piglets to *Clostridium difficile* infection

Einfluss einer Formula-Fütterung auf die Anfälligkeit neugeborener Ferkel gegenüber einer Clostridium difficile Infektion

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Question: *C. difficile* (CD) has been documented as a major cause of uncontrolled ("spontaneous") enteritis outbreaks in neonatal pigs worldwide (1,2). Interestingly, CD and their toxins can be found in up to 100% of neonatal piglets between day 4 and 6 of life without clinical symptoms (3). So far, animal models of CD infection (CDI) have been established mainly in gnotobiotic neonatal piglets (2) making the interpretation and the use for intervention studies difficult. We hypothesized that diet, rearing environment, and time point of infection could affect the predisposition of piglets to CDI.

Methods: In a series of four consecutive trials, a total of 48 neonatal piglets were moved into artificial rearing units at day 1 of life and received a bovine milk-based formula (app. 46% lactose, 20% ether extract, 23% crude protein). In trial 1, twelve piglets (n=4 per group) were infected 48 h after birth with 10^4 or 10^6 cfu CD (Ribotype 078), whereas control piglets (n=4) received a sham inoculum and the experimental period was ten days. In trial 2, piglets were infected (n=6) or not (n=6) with 10^8 cfu CD. Trial 3 was similar to trial 2 but with infection at 6 h of life. In trial 4, piglets in one group (n=6) were treated with antibiotics (clindamycin) 24 h after birth and all piglets (n=12 total) were infected with 10^8 cfu CD after 48 h. All the piglets were housed in pairs in the artificial rearing units. Fecal samples were taken during the experimental period and assessed for CD (by plating on Chrom ID agar plates or quantitative real-time PCR) and CD toxin B (TcdB) levels by ELISA. In trials 2-4, piglets were euthanized 72 h after the infection for identification of gross lesions, histopathological examinations in HE and PAS-AB stained colon tissue and sampling of digesta. Samples from suckling piglets (n=6) at similar age and from the same sows as in trial 3 and 4 were taken for comparison. Data were non-normally distributed and therefore analysed by Kruskal-Wallis test and Mann-Whitney U test. The statistical significance was considered at $P<0.05$.

Results: Control and infected formula-fed animals developed diarrhoea during the first days after birth and no differences in clinical signs or performance were observed ($P>0.05$). High levels of other CD ribotypes (between 5 to 6 log cfu/g and 8 to 9 log 16S rDNA gene copies) and its TcdB could already be detected in the feces of all animals prior to the infection. CD and TcdB occurred much earlier and at higher levels in formula-fed than in suckling piglets and were higher in control as compared to infected animals in trial 3 ($P<0.05$). CD and toxin concentrations peaked in all animals 48 h after the infection. Signs of CDI such as mesocolonic edema and gas-filled distal small intestines were observed after 72 h in all animals in trials 2-4 but not in trial 1. Histological examination revealed cellular infiltrations and loss of goblet cells in the colon of control and infected piglets after 72 h as compared to suckling piglets. Histopathological scores did not differ between controls, infected animals and those that received clindamycin prior to infection. In trial 1, fecal CD and TcdB declined below the detection limit after 10 days suggesting a recovery from CDI after this time.

Conclusions: We demonstrate that artificial rearing and formula feeding predisposes neonatal piglets to colonization by toxigenic CD ribotypes much earlier as compared to suckling piglets, and the infection with a hypervirulent CD ribotype does not exhibit further aggravation. Neither time point of infection or antibiotic pre-treatment showed any additional effect. It appears that maternal factors may play an essential role to prevent CDI in suckling as compared to formula-fed neonatal piglets.

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160.

Influence of drying at low temperatures on composition of nitrogenous compounds in excreta of broilers and caecectomised laying hens

Einfluss von Trocknung bei geringen Temperaturen auf die Zusammensetzung stickstoffhaltiger Inhaltsstoffe von Exkrementen von Broilern und caecectomierter Legehennen

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Excreta collection is a standard technique for different research questions related to animal nutrition. Such research questions include the determination of digestibility of nitrogen (N) or amino acids (AA), and the estimation of N retention. The chemical composition of excreta can change after excretion due to microbial activity or exposition to the environment. The extent of the change in chemical composition of the excreta dried at low temperatures, however, and the impact on related research questions is largely unknown. The aim of this study was, therefore, to investigate the effect of different temperatures and drying durations on selected nitrogenous compounds in excreta of broilers and caecectomised laying hens.

Methods: Seven treatments were investigated. Excreta were frozen immediately after excretion, or dried for 4, 6 and 12 h at one of two temperatures. The drying temperatures were 23 and 33°C in experiment 1 (E1; broilers) and 19 and 29°C in experiment 2 (E2; caecectomised laying hens). When the excreta were collected, 24 broilers were kept in 8 metabolism cages of 3 birds each in E1, and 14 hens were kept in 7 metabolism cages of 2 birds each in E2. The excreta collection lasted for 96 and 72 continuous hours in E1 and E2, respectively. Each cage was defined as one statistical unit. Immediately after excretion, the droppings were mixed, divided in 2 parts, and randomly assigned to 2 of the 7 treatments. The excreta were stored in drying chambers at low airflow during drying and immediately frozen after the respective predefined drying duration.

Table: Effect of storage duration and temperature (19 and 29°C in E1, and 23 and 33°C in E2) on selected characteristics of excreta of laying hens (g/kg dry matter unless otherwise stated)

Temperatur		imme-diately	lower temperature			higher temperature			Pooled SEM
Duration		frozen	4h	6h	12h	4h	6h	12h	
E 1	Dry matter (g/kg)	157^{g}	212^{f}	240^{e}	394^{c}	328^{d}	472^{b}	794^{a}	10.2
	Total N	51.3	50.9	51.0	51.2	50.9	50.7	50.7	0.6
	Ammonia	1.04^{ab}	1.10^{ab}	1.17^{a}	1.10^{ab}	1.14^{ab}	1.00^{b}	0.81^{c}	0.05
E 2	Dry matter (g/kg)	192^{e}	229^{d}	243^{d}	309^{c}	333^{c}	397^{b}	667^{a}	11.0
	Total N	58.6	58.9	58.9	58.9	60.6	57.2	56.8	1.5
	Ammonia	2.82	3.10	2.97	3.07	3.08	2.97	2.99	0.15
	Sum of AA[1]	93.1^{a}	78.2^{c}	78.5^{c}	85.0^{b}	70.4^{d}	73.2	81.0^{c}	1.15

[1]Sum of all proteinogenic AA except for tryptophan; determined by ion-exchange chromatography

Results: The dry matter content of the excreta increased linearly ($P<0.01$) with drying duration in both experiments (Table). Temperature and drying duration had no effect on the total N concentration in the excreta ($P>0.05$). The ammonia concentration was not influenced in E2. In E1, the ammonia concentration was decreased ($P<0.05$) at 12h of drying at 33°C; no differences compared to the undried excreta ($P>0.05$) were observed in the other treatments. The concentration of the sum of AA in the excreta in E2 was highest ($P<0.05$) when the excreta were immediately frozen and decreased until 4h of drying. Then, the AA concentration increased with increasing drying duration.

Conclusions: Drying excreta at low temperatures has no effect on N and ammonia concentration in excreta of caecectomised laying hens and no effect on total N concentration in broilers. These results suggest that drying at low temperature is suitable for experiments investigating these traits. Excreta drying at low temperatures cannot be recommended because the analysed AA concentration in the excreta was influenced to an extent of relevance for AA digestibility studies.

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Campylobacter jejuni infection in chicken of different genetic lines and ages under the influence of dietary lauric acid rich palm kernel fatty acids in a complete diet

Einfluss erhöhter Laurinsäuregehalte im Alleinfutter von Broilern verschiedener genetischer Linien und unterschiedlichen Alters auf den Verlauf einer experimentellen Infektion mit Campylobacter jejuni

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Campylobacteriosis is the most frequently occurring zoonosis in the European Union and infections are often linked to the consumption and handling of poultry meat [1]. Lauric acid is known for its ability for inhibiting culture growth of *Campylobacter jejuni* in vitro [2]. Distilled palm kernel fatty acids (PKFAD) are characterized by a high concentration of lauric acid [3]. The hypothesis of the present study was that a diet rich in lauric acid has the capability to reduce the susceptibility of broiler chickens to an experimental *Campylobacter jejuni* infection.

Methods: In three consecutive trials a total of 450 chickens of different genetic lines and ages (A1 -two weeks of age; A2 - 9 or rather 10-week-old - see table 1) were assigned randomly to 30 groups and reared for 28 days in boxes littered with wood shavings. All groups were fed ad libitum an industrially produced compound feed containing 10 % coarsely ground wheat and monensin-Na as coccidiostat. A total of 15 groups received the diet containing a conventional plant fat mixture as control diet (per kg diet: 12.7 MJ ME, 189 g XP, 81.0 g XL, 6.62 Ca, 5.60 P, 0.82 g Na, 6.46 g K, 0.40 g lauric acid). The other 15 groups were fed the experimental diet in which 5.00 % of the conventional plant fat mixture was replaced by PKFAD (per kg diet: 12.6 MJ ME, 193 g XP, 77.0 g XL, 7.00 Ca, 5.95 P, 0.82 g Na, 7.00 g K, 18.8 g lauric acid). The animals were adapted to the feed for seven days before three animals of every group were experimentally infected with *Campylobacter jejuni* (10^4 cfu/ml). On day 2, 4, 7, 14 and 21 after experimental infection individual cloacal swabs were taken. At dissection caecal content was analysed quantitatively for Campylobacter counts. The microbiological tests were applied in accordance to DIN EN ISO 10272-1. Statistics were done by 1-way-ANOVA and Pearson's chi-squared test ($p < 0.05$).

Results: The body weight at the end of the experiment differed significantly depending on genetics (in g; Ross 308: 2836±422^a, Hubbard: 1859±297^b, Lohmann Dual: 1051±149^c, Lohmann Brown-Classic A1: 595±59.0^d) and age (in g; Lohmann Brown-Classic A1: 595±59.0^b, A2: 1834±137^a). The diet had no influence on the development of the body weight. Two days after experimental infection the Campylobacter prevalence was significantly reduced (19 %) due to the provision of the experimental diet in comparison to the control diet (control: 110/225 positive; experimental: 89/225 positive). In the further course of the infection no significant differences between the feeding groups were seen ($p < 0.05$).

Table 1: Counts of *C. jejuni* (log cfu/g) in caecal content depending on genetic line, diet and age (N=450) at dissection (21 days after experimental infection)

Genetic line	Age categoryat start	Control diet (n=45/line[1])	s	Experimental diet (n=45/line[1])	s	p-value
Ross 308	A1	8.44^{Aa}	±0.76	8.44^{Aa}	±0.69	0.86
Hubbard JA 757	A1	8.38^{Aa}	±0.79	8.49^{Aa}	±0.55	0.93
Lohmann Dual	A1	8.40^{Aa}	±0.61	8.40^{Aa}	±0.53	0.76
Lohmann Brown-Classic	A1	8.62^{Aa}	±0.38	8.52^{Aa}	±0.52	0.44
Lohmann Brown-Classic	A2	6.54^{Ba}	±1.52	6.79^{Ba}	±1.34	0.53

[1] from a total of 450 animals in 448 animals Campylobacter numbers were countable in the caecum content; Different letters within rows and columns indicate significant differences with $p< 0.05$

Conclusion: The prevalence of Campylobacter could be reduced through the experimental diet. However, this effect was limited to the initial phase of infection. At dissection no differences occurred in caecal content depending on the diet. Usually the Campylobacter entry to the animal stock occurs during the last third of fattening (e.g. after the 1st catch). If the Campylobacter entry occurs at this stage of fattening possibly the PKFAD containing diets could offer a chance to reduce the prevalence in the animal group until slaughter and therefore the risk of human campylobacteriosis [4].

1. EFSA Journal 2013. 2013;11(4):3129 ff.; 2. Folia Microbiol (Praha). 2010;55(3):215-20.; 3. Enzyme and microbial technology. 2005;36(5):725-8.; 4. Int J Food Microbiol. 2003;83(1):87-103.

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Effects of diets' physical form as well as of the housing in littered pens on broiler's organ development

Effekte der physikalischen Struktur des Mischfutters und der Haltung auf Einstreu auf die Entwicklung von Organen des Verdauungstrakts bei Masthähnchen

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Already in the 1970th authors found a disease characterized by a loss of the typical form of the isthmus between the proventriculus and the gizzard of broilers called proventriculus dilatation [1]. In particular cage-reared chickens with small gizzards were affected. These findings led to the hypothesis that the addition of 10% whole wheat to a pelleted diet and the housing on litter increased gizzard weight and reduced prevalence of isthmus malformation (IM) in broilers.

Methods: Two trials with four treatments (T) in each and 3 replicates (pens) per treatment (à 20 broilers, 8 days old, Ross 308, both sexes) were conducted. For treatment I birds were housed littered (wood shavings, 1kg/m^2) whereas in treatment II a floor heating was installed improve litter quality. For treatment III boxes were divided into a littered and a slatted half whereas for treatment IV the floor was fully slatted but a sand bath (900cm^2) was provided. In trial 1 wheat based pelleted (3mm) grower and finisher diets mixed with 10% whole wheat were fed. In trial 2 the 10% whole wheat offered in trial 1 were ground and included into the pellets. Water and feed were offered ad libitum. During days 10-14 of life enrofloxacin (10mg/kg) was administered via drinking water. On day 23 (d23) of life 8 birds per box and on d36/37 6 birds per group and day were dissected. Beside the measurement of body weight, the gizzard and pancreas weights of 5 (d23) and 7-8 birds per box (d36 and d37) were determined. Additionally the form of the isthmus between the proventriculus and the gizzard was characterized by macroscopical scoring. Statistical analyses were performed with SAS® software (Cary, NC, USA; PROC NPAR1WAY, WILCOXON-test).

Results: The prevalence of isthmus malformation differed between the trials. The birds fed the diet with 10% of whole wheat (trial 1) showed markedly lower prevalence. In contrast to WITTE [2] already on d23 of life dilatation of isthmus between proventriculus and gizzard was observed (24.5% of birds on d23; 18.0% of birds on d36/37; data not shown). There was no link between the occurrence of isthmus malformation and the offer of litter. Regarding the gizzard and pancreas weights per kg BW there were similar tendencies in comparison to the trials. The birds fed the diet including the whole wheat showed higher relative organ weights independent of the day of dissection. The relative gizzard weight was highest in birds housed on litter (I) followed by birds housed in boxes with floor heating, birds housed in partly slatted and finally birds housed on full slatted floor.

	T I	**T II**	**T III**	**T IV**	**Trial 1**	**Trial 2**
IM prevalence						
-d23+d37/37 (%)	37.4	24.0	33.7	42.0	23.4	44.6
Gizzard (g/kg BW)						
-d23	19.3±4.63^a	18.9±4.69^a	18.8±4.86^a	17.3±4.85^a	22.6±2.50^a	14.5±2.37^b
-d36/37	10.4±2.34^a	10.2±2.22^a	10.2±2.41^a	9.31±1.95^a	11.5±2.06^a	8.74±1.50^b
Pancreas (g/kg BW)						
-d23	2.45±0.42^a	2.29±0.31^a	2.38±0.33^a	2.26±0.36^a	2.54±0.30^a	2.15±0.31^b
-d36/37	1.50±0.23^a	1.45±0.25^a	1.55±0.26^a	1.48±0.25^a	1.56±0.27^a	1.43±0.21^b

There was no correlation between the relative weight of the gizzard and the occurrence of IM.

Conclusion: As stated in the literature a coarse physical form of the diet (here achieved by the addition of 10% whole wheat to the pelleted diet) seem to reduce the prevalence of IM and to increase the gizzard and pancreas weights. Furthermore there was a trend for increased gizzard weights when litter material was available. The numerical tendency might be caused by the intake of indigestible particles from wood shavings. Beside the effects of the diet, the litter material might be an influencing factor regarding the development of the gastrointestinal tract.

[1] RIDDELL, C. (1975): Avian Dis. 20, 442-445; [2] WITTE, M. (2012): Univ. of Vet. Med, Diss., Hannover

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Intestinal luminal pH is associated with worm expulsion in chickens

Intestinale-luminale pH ist assoziiert mit der Wurm-Expulsion beim Huhn

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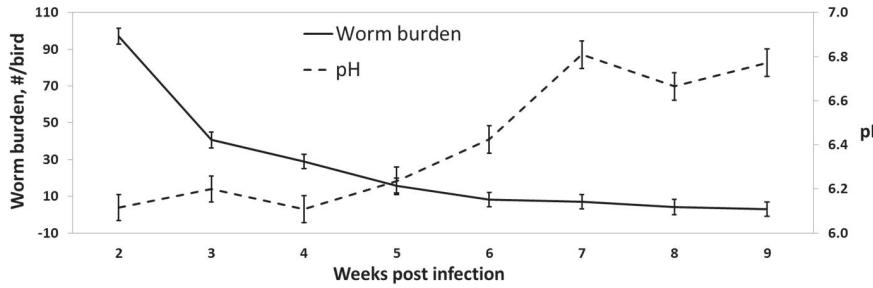
Infections of chickens with the nematodes *Ascaridia galli* and *Heterakis gallinarum* are re-emerging in the European farms. Intestinal pH is an important factor that may affect establishment, survival and fecundity of nematodes in their specific predilection sites. Different chicken genotypes differ in intestinal pH and microbial colonization. Thus, the aim of this study was to investigate whether luminal pH of the small intestine, the predilection site of *A. galli*, is associated with the worm burdens of experimentally infected chickens in three divergent genotypes, developed either for meat or egg production or for both purposes.

Material and Methods: A total of 576 male 1-week-old chicks of three genotypes namely Ross-308 (R), Lohmann Dual (LD) and Lohmann Brown+ (LB+) were used. The birds were either infected with 500 embryonated eggs of *Ascaridia galli* and *Heterakis gallinarum* or kept as uninfected controls. Starting from 2 weeks post infection (wpi) on, randomly selected birds of each genotype were necropsied at weekly intervals until an age of 10 weeks (i.e. 9 wpi). Infection intensity of the chicks with *A. galli* was quantified, and the luminal pH at the Meckel´s diverticulum was measured. Worm burden data were log-transformed. The data were subjected to analyses of variance (Proc GLM, SAS) considering effects of infection, genotype, wpi and all possible interactions among these 3 factors, plus run and pen effects. Correlation analysis was used to investigate relationships between worm burden and pH values in the infected birds.

Results: Overall average worm counts per bird decreased ($P<0.001$) over time linearly from 97 ± 4.3 worms (LSM ± SE) at 2 wpi to 3 ± 3.9 worms at 9 wpi (Fig. 1). Although the genotypes tended to differ in overall average worm burdens ($P<0.10$), there was no time dependent change among the three genotypes ($P>0.05$). Infected birds had a lower ($P<0.001$) intestinal pH as compared to their uninfected counterparts, although there was a time dependent linear increase in the pH throughout the studied period (Fig. 1; $P<0.001$). There was a difference among the genotypes for the intestinal pH ($P=0.032$), with LB+ having lower values than R ($P<0.05$) and tending to differ from the LD ($P<0.10$), whilst no difference was quantified between R and LD ($P>0.05$). No significant interaction was quantified among the effects of infection, genotype and time ($P=0.440$), indicating constant differences between infected and uninfected birds of all genotypes throughout the studied period. A moderate-negative correlation ($r=-0.45$; $p=0.003$) between worm burden and intestinal pH was quantified only at 2 wpi, while no significant correlation could be determined at later time points.

Figure 1: Time dependent alterations in the worm burdens and intestinal-luminal pH in chickens.



Conclusions: The linear decrease in the worm counts of the birds indicates the existence of an effective defense mechanism responsible for worm expulsion in all 3 genotypes. The worm expulsion was accompanied by the increasing intestinal pH over time. Worm burdens and intestinal pH at the earlier stages of infection (i.e. 2 wpi) correlated negatively, and pH values of infected birds were lower as compared to uninfected birds throughout the study period. Thus, we conclude that the intestinal pH is associated with worm expulsion mechanisms.

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What is the milk composition of the Indian rhinoceros (*Rhinoceros unicornis*) and how does it change within one year?

Wie ist die Milch vom Panzernashorn (Rhinoceros unicornis) zusammengesetzt, und wie ändert sich die Zusammensetzung innerhalb einer Laktation von einem Jahr?

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Question: The aim of this study was to analyze the nutrient composition of Indian rhinoceros' milk (*Rhinoceros unicornis*), to study its changes over the course of a year and to compare the results to previous studies on rhinoceros' milk as well as other hindgut fermenters, elephant and horse.

Methods: Milk samples were collected from two Indian rhinoceros cows from Zoo Basel. From one cow, "Quetta", three milk samples were taken and analyzed (colostrum, milk one week and two weeks post-partum). From the other cow "Ellora", samples were collected in regular intervals over the course of one year and fifteen were selected for analyzes (from colostrum to 13 months post-partum). In the milk samples, the following parameters were measured after lyophilisation: Dry matter (DM), crude ash (CA), crude protein (CP), crude fat (EE), lactose, calcium (Ca), phosphorus (P), magnesium (Mg), fatty acids (FA) and gross energy (GE). DM, CA, CP and EE were determined with proximate analysis according to Naumann & Bassler (1) and nitrogen-free extract (NfE) was subsequently calculated. Lactose was analyzed with infrared spectroscopy and an enzymatic method, Ca, P and Mg with an autoanalyzer, FA with gas chromatography and GE with bomb calorimetry. Changes between the lactation periods were statistically evaluated with an analysis of variance (ANOVA).

Results: The composition of Elloras' colostrum was: 13.8% DM (on whole-milk basis), 4.8% CA, 61.8% CP, 0.7% EE, 32.6% NfE, 26.7% lactose, 0.59% Ca, 0.54% P, 0.2% Mg (on DM basis (DMB)), 20.3 MJ GE/kg DM. Elloras' sample collected 13 months post-partum averaged 8.0% DM (on whole-milk basis), 3.6% CA, 16.3% CP, 1.8% EE, 78.3% NfE, 84.7% lactose, 0.54% Ca, 0.48% P, 0.09% Mg (DMB), 17.43 MJ GE/kg DM. Capric acid (C10:0), lauric acid (C12:0), palmitic acid (C16:0), oleic acid (C18:1n9c) and linoleic acid (C18:2n6c) were the main FA in Ellora's and Quetta's samples. Regarding the FA profile, the milk of the Indian rhinoceros is similar to that of the African elephant.

Conclusion: Crude analysis showed that the milk of the Indian rhinoceros contains very low fat and protein levels but high lactose concentrations, which is comparable to the milk composition of other rhinoceros species and horses, however not to elephants. Nevertheless, with higher fat levels, slightly lower lactose and Mg values and a different FA profile, horse's milk is not an optimal substitute for the rhinoceros' calves, unless it is supplemented with a fat source that has a similar FA profile to rhinoceros milk and additional lactose.

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Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology

Mitteilungen des Ausschusses für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie

Equations for predicting metabolisable energy and digestibility of organic matter in forage legumes for ruminants

Gleichungen zur Schätzung der Umsetzbaren Energie und der Verdaulichkeit der Organischen Substanz von Grobfutterleguminosen für Wiederkäuer

July 2016

1 Introduction:

In recent years, the cultivation of forage legumes such as lucerne, red and white clover has become more important to obtain roughage sources. This is particularly relevant not only for supplying ruminants with farm-produced protein, but also for improving the supply of feed to induce an adequate structural fibre effect. When forage legumes are included in ration planning, its energy value must be adequately predicted. Evaluation of the data described below had suggested that the metabolisable energy (ME) of forage legumes cannot be predicted with sufficient precision based on the equations recommended for predicting the ME of grass and grass products (GfE 2008), and prediction equations were therefore derived specifically for forage legumes. This work was based on data sourced from digestibility experiments conducted in various institutions and regions in Germany and Switzerland with lucerne, sanfoin, red and white clover with wide variations in chemical composition (Losand et al. 2013). Equations for predicting the digestibility of organic matter (DOM) were additionally developed.

2 Data and procedures:

A total of 89 data sets obtained from digestibility experiments conducted in seven institutions were used in the evaluation (Table 1). The digestibility of nutrients in freshly harvested forage, silage, hay and dried forage was determined in sheep according to the guidelines of the Society of Nutrition Physiology (GfE 1991) over a period extending from 2000 to 2014. The ME was calculated from the digestible crude nutrients using the following equation (GfE 1995, 2001):

ME (MJ/kg)	= 0.0312	• digestible crude fat(g/kg)
	+ 0.0136	• digestible crude fibre(g/kg)
	+ 0.0147	• digestible organic residue (digestible organicmatter
		- digestible crude fat - digestible crude fibre) (g/kg)
	+ 0.00234	crude protein(g/kg)

Abbreviations used: **ADFom** = acid detergent fibre expressed exclusive of residual ash; **ADL** = acid detergent lignin; **aNDFom** = amylase-treated neutral detergent fibre expressed exclusive of residual ash; **CA** = crude ash; **CF** = crude fibre; **CL** = crude fat; **CP** = crude protein; **DM** = dry matter; **DOM** = digestibility of organic matter; **ESOM** = enzyme soluble organic matter; **GE** = gross energy; **GP** = gas production; **ME** = metabolisable energy; **NEL** = net energy for lactation; **NfE** = nitrogen-free extracts; **OM** = organic matter; $\mathbf{R^2}$ = coefficient of determination; **s%** = coefficient of variation; $\mathbf{v_R}$ = residual variance

Table 1: Number of data sets by origin, type of preservation and cut number

		Type of preservation			Cut number		
Origin/ Institution	Total	Fresh	Silage	Hay/dried forage	1st cut	Regrowth	Not stated
ALP Posieux	20	7	4	9	7	11	2
Bingen	8		8		4	4	
Dummerstorf	35	15	12	8	9	24	2
Grub	16		16		6	6	4
Halle	1		1				1
Köllitsch	4	2		2			4
Kleve	5			5			5

The full data material from 89 data sets was used in the mathematical derivation of the estimating equations. For validation purposes, the derived equations were either applied separately to the categories forage, silage and hay/dried forage, and 1st cut, regrowth and 'Not stated' respectively, or to the full data material. Table 2 summarises the data from the comprehensive characterisation of the material.

Table 2: Composition[1]), *in vitro* criteria, digestibility of organic matter and calculated ME content of total material (mean, standard deviations, coefficient of variation s%, minimum and maximum).

		n	Mean	s	s%	Min.	Max.
Crude ash		89	120	34.5	28.7	69	282
Crude protein		89	194	34.9	18.0	112	276
Crude fat		89	23	7.7	34.1	5	38
Crude fibre	g/kgDM	89	265	64.0	24.1	114	382
aNDFom		84	402	89.8	22.4	185	600
ADFom		84	317	62.9	19.8	141	412
Lignin (ADL)		21	63	10.8	17.2	36	83
Gas production	ml/200mgDM	54	41.6	5.8	14.0	29.7	51.3
ESOM	g/kgDM	82	597	64.7	10.8	459	741
DOM	%	89	67.5	7.0	10.4	53.5	82.8
ME	MJ/kgDM	89	9.21	0.9	9.3	7.5	11.3

1) Silage values except gas production corrected for e losses of volatile substances during drying.

With coefficients of variation between 20 and 24% for the fibre fractions, of 18% for crude protein, of 34% for crude fat, and of approximately 10% for DOM and ME contents respectively, the data exhibited sufficient variance for deriving regression equations. The lignin content (determined as acid detergent lignin/ADL) was only stated in some of the data sets, and this fraction was therefore not taken into account in the regression calculations. However, the relevant values have been included in Table 2 for reasons of completeness and as a basis for possible future updates. The values for crude fibre contents are also stated in order to characterise the material in more detail, and they are required for calculating the reference values for ME. However, crude fibre was not taken into account as a variable in deriving the estimating equations. The estimating equations were derived from the ME contents, the contents of nutrients and enzyme soluble organic matter (ESOM), and the gas production (GP) of the organic matter. Validation was, however, based on the converted ME content relative to dry matter, and standard error and bias are also stated on this basis.

As a first step, various estimating equations for predicting ME contents were derived and subsequently compared based on their coefficient of determination (R^2), residual variance (v_R) and bias. Initially it was intended to derive a single estimating equation that would be valid for all of the forage legumes. However, this did not yield satisfactory results with all types of preservation and cut numbers, and separately derived estimating equations were therefore examined for the 1st cutand regrowth, similar to earlier approaches for grass and forage products (GfE 1998). As a third step, the derived estimating equations were validated using the full data pool, separated by type of preservation and cut number. The equations were derived using the ***Proc Reg*** procedure of the SAS® statistical software package with stepped parameter selection. The following variables were considered, using a constant additive value: crude protein, crude fat, ADFom or aNDFom respectively, ESOM and GP, relative to the content in organic matter. Conceptionally, variants were first calculated using one of the two *in vitro* parameters and then also used together in another derivation due to the relatively low correlation between ESOM and GP ($r = 0.64$). Only variables with a level of significance of $p < 0.15$ were taken intoaccount.

3 Results:

3.1 Regression equations for calculating ME

Table 3 summarises the results of the evaluation. The inclusion of aNDFom was not helpful in any case, as the level of significance of the regression coefficient was consistently substantially below that for ADFom. Equations 1-3 have been derived based on the full data pool without special consideration of the cut number. However, these equations are only comparable to a limited degree in terms of the accuracy of the estimate they allow (R^2, v_R), as they are each based on data pools of different sizes. With an already relatively high coefficient of determination of $R^2 = 0.84$ and a low residual variance v_R of 0.47 or 0.50 MJ/kg OM respectively, the reliability of the estimated ME content of forage legumes is improved even further by using both ESOM and GP together. This also applies similarly to the separate ME content estimates for the 1st cut and regrowth and the category without stated cut number respectively. The materials for which the cut number was not stated were counted among the regrowth cuts in the evaluation and not among the 1stcut.

Table 3: Coefficient of determination (R^2) and residual variance (v_R) of the derived estimating equations for the ME content of organic matter

Equation No.	n	Variables	Cut number	R^2	v_R
ME_1	81	ADFom, CP, CL, ESOM	Not differentiated	0.84	0.47
ME_2	54	ADFom, CP, CL, GP	Not differentiated	0.84	0.50
ME_3	53	ADFom, CP, CL, ESOM, GP	Not differentiated	0.89	0.43
ME_1_1	22	ADFom, CP, CL, ESOM	1	0.90	0.36
ME_2_1	17	ADFom, CP, CL, GP	1	0.89	0.42
ME_3_1	16	ADFom, CP, CL, ESOM, GP	1	0.89	0.36
ME_1_2	58	ADFom, CP, CL, ESOM	Regrowth and not	0.85	0.46
ME_2_2	36	ADFom, CP, CL, GP	Regrowth and not	0.86	0.50
ME_3_2	36	ADFom, CP, CL, ESOM, GP	Regrowth and not	0.91	0.41

3.1.1 Validation of the prediction equations for ME

Validation was performed for the full data pool, separately for the categories forage, silage and hay, and separately for 1st cut, regrowth and data material without indication of cut number (Table 4), using the leave-one-out cross-validation approach. Independent validation was not possible due to the limited number of samples in the various categories following differentiation. It was noted that the selection of the *in vitro* parameter is irrelevant for the accuracy of the estimate, as the standard error remains approximately the same. Where analytical results are available for both *in vitro* parameters, the accuracy of estimate can be improved further. In terms of the type of preservation, the robustness achieved with a general equation that does not take this aspect into account was only given for ESOM. Inclusion of the GP parameter for the data pool (which was not, however, identical) revealed that the application of the estimating equation results in substantial underestimation for fresh forage. However, the considerable, general underestimate of the 1st growth ME content by more than 0.2 MJ ME/kg DM that resulted from the use of equations 1-3 was more remarkable. Thus, there were differences between crops from the 1st cut and from regrowths throughout the year, which are not represented by commonly used analytical variables, even when the two *in vitro* digestibility characteristics are included. Where estimating equations are derived separately for 1st cut and regrowth materials, this biased deviation is largely neutralised. However, even in this case a systematic underestimate of the ME content of forage persists when GP isused.

Table 4: Standard error (%) and bias in the validation of the derived estimatingequationsfor calculating ME content (MJ ME/kg DM), differentiated by type of preservation and cut number

Equation	Parameter	All	Type of preservation			Cut number		
			Fresh	Silage	Dried	1	Regrowth	Not stated
1	n	81	24	34	23	23	42	16
	Mean	9.22	9.78	9.12	8.78	9.55	9.22	8.73
	% standard error	4.4	5.2	4.3	3.3	3.5	4.4	3.7
	Bias	0.00	-0.04	-0.04	0.11	-0.25	0.05	0.24
2	n	54	13	19	22	18	24	12
	Mean	9.11	9.90	8.85	8.88	9.41	9.06	8.78
	% standard error	4.7	2.5	5.3	4.6	3.7	4.4	5.4
	Bias	0.00	-0.28	0.03	0.14	-0.23	0.06	0.23
3	n	53	13	19	21	17	24	12
	Mean	9.11	9.97	8.86	8.80	9.45	9.07	8.71
	% standard error	4.0	3.4	4.6	3.4	3.6	4.0	3.2
	Bias	0.00	-0.20	0.04	0.09	-0.20	0.06	0.16
1_1/1_2	n	81	24	34	23	23	42	16
	Mean	9.21	9.77	9.16	8.76	9.80	9.13	8.63
	% standard error	4.3	5.0	4.0	3.9	3.6	4.6	4.3
	Bias	0.01	-0.03	-0.02	0.08	0.01	-0.05	0.14
2_1/2_2	n	54	13	19	22	18	24	12
	Mean	9.15	9.92	8.94	8.92	9.70	8.97	8.70
	% standard error	5.6	2.7	7.0	4.9	6.1	47.6	6.7
	Bias	0.04	-0.26	0.07	0.18	0.01	-0.03	0.16
3_1/3_2	n	53	12	20	21	17	24	12
	Mean	9.11	9.97	8.93	8.78	9.63	8.96	8.65
	% standard error	4.5	4.0	5.1	3.9	4.8	4.3	4.3
	Bias	0.00	-0.21	0.05	0.07	-0.01	-0.04	0.10

Figure 1 shows the differences between the ME content estimates based on equation 1 and the values calculated from digestible crude nutrients. Based on equation 1, the residual variance of the estimated ME content remains about constant across almost the entire range of variation. Figure 2, in contrast, clearly indicates the negative deviations of the ME content estimates of 1st cut material based on equation 1. In equation 1, the underestimated ME content is offset by the tendency towards over- estimating materials without stated cut number. Where 1st cut materials are evaluated separately, this estimate error for 1st cut materials and materials without stated cut number is no longer present (Figure 3).

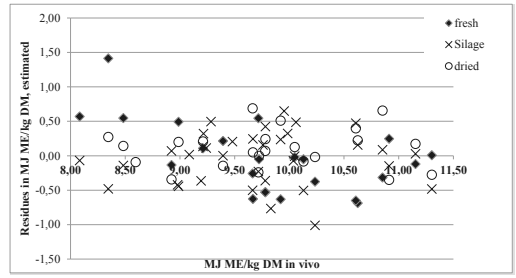

Figure 1: Differences between estimated ME contents and ME contents determined in digestibility trials with sheep by type of preservation (equation ME_1)

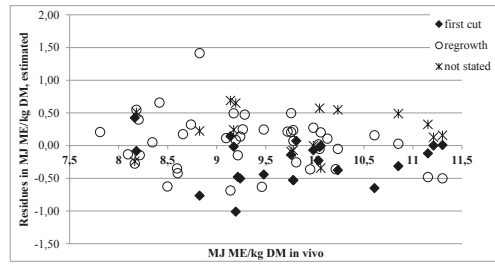

Figure 2: Differences between estimated ME contents and ME contents determined in digestibility trials with sheep by cut number (equation1)

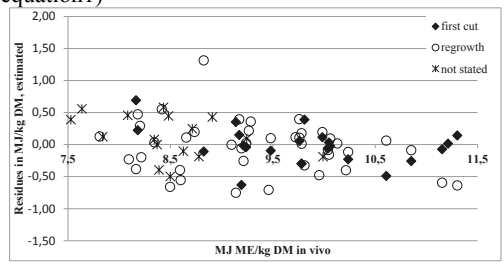

Figure 3: Differences between estimated ME contents and ME contents determined in digestibility trials with sheep by cut number (equations ME_1_1 and ME_1_2)

3.1.2 Recommended equations for predicting ME in forage legumes

In line with the procedure for grass and maize products (GfE 2008) and compound feeds for ruminants (GfE 2009), the Committee recommends the use of two alternative equations for predicting ME in forage legumes, taking into account either ESOM or GP (Table 5). However, with both alternatives a separate equation needs to be used for the 1st cut in order to improve the accuracy of the estimate. A different equation is consequently recommended for regrowth. If no information is provided on the cut number, the equation for regrowths should be used; this results in 1st cut samples not marked as such being underestimated by about 0.3 MJ ME/kg DM. In order to avoid this systematic error it is therefore important that samples sent in for evaluation are clearly and routinely marked with the cut number.

Table 5: Recommended equations for predicting the ME content of organic matterinforage legumes

Based on ESOM					
First cut			Regrowth and without stated cut number		
ME =	11.91		ME =	9.83	
	- 0.01034	• ADFom		- 0.01010	• ADFom
	+ 0.00389	• CP		+ 0.00039	• CP
	+ 0.01870	• CL		+ 0.00802	• CL
	+ 0.00191	• ESOM		+ 0.00571	• ESOM
R^2 =	0.904		R^2 =	0.852	
v_R =	0.36		v_R =	0.46	
ME in MJ/kg OM; CP, CL, ADFom, ESOM in g/kg OM					

Based on gas production					
First cut			Regrowth and without stated cut number		
ME =	12.49		ME =	11.09	
	- 0.01140	• ADFom	- 0.01040	• ADFom	
	+ 0.00425	• CP	+ 0.00497	• CP	
	+ 0.02690	• CL	+ 0.00750	• CL	
	+ 0.01683	• GP	+ 0.0351	• GP	
R^2 =	0.891		R^2 =	0.862	
v_R =	0.42		v_R =	0.50	
ME in MJ/kg OM; CP, CL, ADFom in g/kg OM; GP in ml/200 mg OM					

The following equation was then used to convert values into ME content in dry matter for specific samples for evaluation based on their organic matter content:

ME (MJ/kg DM) = ME (MJ/kg OM) • [1000 – CA (g/kg DM)]/1000

For dairy cows, net energy lactation (NEL) can be calculated from ME as follows, taking into account gross energy (GE) metabolisability (q) according to GfE (2001):

NEL (MJ) = 0,6 [1 + 0.004 (q – 57)] ME (MJ), with q = ME/GE • 100

If the GE content has not already been measured by bomb calorimetry, it must initially be determined using the following equation:

GE (MJ/kg DM) = 0.0239 • CP (g/kg DM) + 0.0398 • CL (g/kg DM) + 0.0201 • CF (g/kg DM + 0.0175 • NfE (g/kg DM)

For this calculation, the content of crude fibre and N-free extracts (NfE) must be known. If this is not the case, ME can be converted into NEL contents from the following, simplified equation (Weißbach et al. 1996):

NEL (MJ/kg DM) = ME [0.46 + 12.38 • ME / (1000 – CA)], with: ME in MJ/kg DM; CA in g/kg DM

3.2 Regression equations for predicting DOM

Crude protein, crude fat, ADFom, aNDFom and at least one *in vitro* criterion were also used to derive the estimating equations for DOM. The calculations were made using concentrations in organic matter. Parameters were excluded as being insignificant if their regression coefficients did not reach a significance level of $p < 0.15$. Similar to the approach taken in predicting ME, a single equation was initially derived for all types of preservation and cut numbers. A separate equation was then derived in a second step for 1st cut materials. Table 6 summarises the results of the regressionanalyses.

T**able 6:** Coefficient of determination ($R^{2)}$ and residual variance (v_R) of thederivedestimating equations for calculating digestibility of organic matter (in%)

Equation No.	n	Variables	Cut number	R^2	v_R
DOM_1	81	ADFom, CP, ESOM	Not differentiated	0.82	3.17
DOM_2	54	ADFom, GP	Not differentiated	0.82	3.37
DOM_3	53	ADFom, ESOM, GP	Not differentiated	0.87	2.93
DOM_1_1	22	ADFom, ESOM	1	0.89	2.31
DOM_2_1	17	ADFom, GP	1	0.88	2.76
DOM_3_1	16	ADFom, ESOM, GP	1	0.89	2.72
DOM_1_2	58	ADFom ESOM	Regrowth and not stated	0.81	3.23
DOM_2_2	36	ADFom, GP	Regrowth and not stated	0.83	3.36
DOM_3_2	36	ADFom, ESOM, GP	Regrowth and not stated	0.88	2.91

This table shows a clear reduction in the number of independent variables compared to the ME contents estimate (Table 3), which is associated with a higher weighting of the *in vitro* parameters for predicting DOM compared to predicting ME. By using both *in vitro* criteria, the accuracy of the estimate could be somewhat improved compared to the alternative use of a single criterion only.

3.2.1 Validation of the prediction equations for DOM

Validation was again performed based on the entire data pool and the sub-categories (Table 7) using the leave-one-out cross-validation approach. As with the approach taken in predicting ME, the use of only one equation in estimating DOM across all cut numbers and types of preservation consistently results in biased

negative deviations of 1.5 to 1.8 percentage points for 1st cut materials and positive deviations for materials without stated cut numbers, above all. There are additionally inaccuracies in differentiating DOM in fresh and dried crops when using the GP equation (equation DOM_2). Where estimating equations are derived separately for 1st cut and regrowth materials, there is no such biased deviation (equations DOM_1_1+2 to DOM_3_1+2; Figure 4). However, even in this case a biased underestimate persists for forage when GP is used.

Table 7: Standard error and bias in applying the derived equations for predicting digestibility of organic matter (DOM, %) based on the complete data pool and onsub-categories

Equation	Parameter	All	Type of preservation			Cut number		
			Fresh	Silage	Dried	1	Regrowth	Not stated
DOM_1	n	81	24	34	23	23	42	16
	Mean	67.7	72.0	66.1	65.6	69.3	68.6	63.2
	% standard error	4.6	5.6	4.2	3.7	3.4	4.7	4.5
	Bias	-0.01	-0.53	0.11	0.37	-1.68	0.35	1.44
DOM_2	n	54	13	19	22	18	24	12
	Mean	67.3	74.4	63.4	67.1	68.4	68.4	63.6
	% standard error	4.9	3.0	5.3	4.8	4.0	4.5	5.8
	Bias	0.00	-2.29	0.07	1.18	-1.69	0.48	1.58
DOM_3	n	53	13	19	21	17	24	12
	Mean	67.3	74.6	63.7	66.0	68.6	68.4	63.2
	% standard error	4.2	3.7	4.9	3.5	3.5	4.0	4.7
	Bias	0.01	-1.73	0.80	0.36	-1.47	0.46	1.17
DOM_1_1/ DOM_1_2	n	81	23	35	23	23	42	16
	Mean	67.7	72.1	66.3	65.4	71.0	67.8	62.6
	% standard error	4.7	5.5	4.4	4.2	3.6	4.9	5.5
	Bias	-0.02	-0.50	0.20	0.1	0.04	-0.39	0.83
DOM_2_1/ DOM_2_2	n	54	12	20	22	18	24	12
	Mean	67.4	74.9	63.5	66.7	70.0	67.6	62.9
	% standard error	5.0	3.6	5.1	5.4	4.4	4.9	6.2
	Bias	-0.02	-1.77	0.15	0.88	-0.06	-0.34	0.85
DOM_3_1/ DOM_3_2	n	53	12	20	21	17	24	12
	Mean	67.1	75.0	63.7	65.8	69.6	67.6	62.4
	% standard error	4.4	4.1	4.3	4.4	3.7	4.4	5.4
	Bias	-0.20	-1.72	0.31	0.18	-0.46	-0.32	0.4

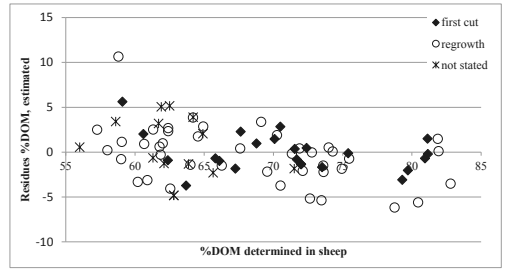

Figure 4: Residues of the estimated digestibility of organic matter (DOM) basedonESOM, compared to values determined in digestibility trials with sheep, by cut number (equations DOM_1_1 and DOM_1_2)

3.2.2 Recommended equations for predicting DOM in foragelegumes

The Committee also recommends the use of two alternative equations for predicting DOM in forage legumes, using ESOM and GP and differentiating by cut number (Table 8). According to both recommendations, a separate equation should be used for the 1st cut, and the relevant regrowth equation should be used for regrowth. The equation for regrowth should be used where no information is available on the cut number. Applying the second equation to 1st cuts that are not marked as such results in an underestimate of digestibility by about 2.5 percentagepoints.

Table 8: Recommended equations for predicting DOM in forage legumes

Based on ESOM					
First cut			Regrowth and without stated cut number		
DOM =	81.71		DOM =	70.77	
	- 0.0711	• ADFom		- 0.0683	• ADFom
	+ 0.0195	• ESOM		+ 0.0302	• ESOM
R^2 =	0.892		R^2 =	0.806	
v_R =	2.31		v_R =	3.23	
DOM in %; ADFom ESOM in g/kg OM;					

Based on gas production					
First cut			Regrowth and without stated cut number		
DOM =	95.72		DOM =	77.90	
	- 0.0859	• ADFom		- 0.0711	• ADFom
	+ 0.0964	• GP		0.2997	• GP
R^2 =	0.859		R^2 =	0.832	
v_R =	2.813		v_R =	3.36	
DOM in %; ADFom in g/kg OM; GP in ml/200 mg OM					

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Gleichungen zur Schätzung der Umsetzbaren Energie und der Verdaulichkeit der Organischen Substanz von Grobfutterleguminosen für Wiederkäuer

Juli 2016

1 Einführung:

In den vergangenen Jahren hat der Anbau reiner Bestände von Leguminosen wie Luzerne, Rot- und Weißklee zur Grobfuttergewinnung an Bedeutung gewonnen. Dies ist vor allem für eine wirtschafts-eigene Proteinversorgung der Wiederkäuer, aber auch zur Verbesserung der Versorgung mit strukturwirksamen Grobfuttermitteln relevant. Bei der Verwendung von Grobfutterleguminosen in der Rationsplanung ist eine Einschätzung ihres Energiewertes notwendig. In Auswertungen des Datenmaterials, das nachfolgend beschrieben wird, hatte sich ergeben, dass bei Anwendung der Gleichungen, die zur Schätzung der Umsetzbaren Energie (ME) für Gras und Grasprodukte empfohlen werden (GfE 2008), eine hinreichend genaue Schätzung der ME von Grobfutter-leguminosen nicht möglich war. Es wurden daher Schätzgleichungen speziell für Grobfutterleguminosen abgeleitet. Hierzu standen Daten aus verschiedenen Institutionen und Regionen Deutschlands sowie aus der Schweiz zur Verfügung, die aus Verdaulichkeitsversuchen mit Luzerne, Rot- und Weißklee sowie Esparsette stammen und deren Inhaltsstoffe über einen sehr weiten Bereich variierten (Losand et al. 2013). Unabhängig von der ME-Schätzung wurden auch Gleichungen zur Schätzung der Verdaulichkeit der Organischen Substanz (VQOS) abgeleitet.

2 Daten und Vorgehensweise:

Für die Auswertung wurden insgesamt 89 Datensätze aus Verdaulichkeitsversuchen verwendet, die in sieben Versuchseinrichtungen durchgeführt wurden (Tab. 1). Die Verdaulichkeit der Nährstoffe im frisch geernteten Grünfutter, in Silagen, Heu und Trockengrün wurde nach den Richtlinien der Gesellschaft für Ernährungsphysiologie (GfE 1991) unter Nutzung von Hammeln im Zeitraum von 2000 bis 2014 bestimmt. Die Berechnung der ME erfolgte aus den verdaulichen Rohnährstoffen unter Verwendung der folgenden Gleichung (GfE 1995, 2001):

ME (MJ/kg)	= 0.0312	• verdauliches Rohfett (g/kg)
	+ 0.0136	• verdauliche Rohfaser (g/kg)
	+ 0.0147	• verdaulicher Organischer Rest (verdauliche Organische Substanz – verdauliches Rohfett – verdauliche Rohfaser) (g/kg)
	+ 0.00234	• Rohprotein (g/kg)

Verwendete Abkürzungen: **ADFom** = Säure-Detergenzien-Faser nach Veraschung; **ADL** = Säure-Detergenzien-Lignin; **aNDFom** = Neutral-Detergenzien-Faser nach Amylasebehandlung und Veraschung; **B** = Bestimmtheitsmaß; **ELOS** = Enzymlösliche Organische Substanz; **Gb** = Gasbildung; **GE** = Bruttoenergie; **ME** = Umsetzbare Energie; **NEL** = Nettoenergie-Laktation; **NfE** = Stickstofffreie Extraktstoffe; $\mathbf{s_R}$ = Reststreuung; **VQOS** = Verdaulichkeit der Organischen Substanz; **TM** = Trockenmasse; **XA** = Rohasche; **XF** = Rohfaser; **XP** = Rohprotein; **XL** = Rohfett

Tabelle 1: Anzahl von Datensätzen nach Herkunft, Konservierungsart und Aufwuchsnummer

Herkunft	Konservierungsart				Aufwuchsnummer		
	Gesamt	Frisch	Siliert	Getrocknet	1. Aufwuchs	Folgeaufwüchse	Ohne Angabe
ALP Posieux	20	7	4	9	7	11	2
Bingen	8		8		4	4	
Dummerstorf	35	15	12	8	9	24	2
Grub	16		16		6	6	4
Halle	1		1				1
Köllitsch	4	2		2			4
Kleve	5			5			5

Für die mathematische Ableitung der Schätzgleichungen wurde das gesamte Datenmaterial von 89 Datensätzen verwendet. Für die Validierung wurden die abgeleiteten Gleichungen auf die Kategorien Grünfutter, Silage und Heu/Trockengrün sowie 1. Aufwuchs, Folgeaufwüchse bzw. „ohne Angabe“ jeweils getrennt oder auf das gesamte Datenmaterial angewandt. In der Tabelle 2 sind die Daten zur näheren Charakterisierung des Materials zusammengefasst.

Tabelle 2: Inhaltsstoffe[1], *in vitro*-Kriterien, Verdaulichkeit der organischen Substanz und berechneter ME-Gehalt für das Gesamtmaterial (Mittelwert, Standardabweichung s, Variationskoeffizient s%, Minimum und Maximum)

		n	Mittelwert	s	s%	Min.	Max.
Rohasche		89	120	34.5	28.7	69	282
Rohprotein		89	194	34.9	18.0	112	276
Rohfett		89	23	7.7	34.1	5	38
Rohfaser	g/kg TM	89	265	64.0	24.1	114	382
aNDFom		84	402	89.8	22.4	185	600
ADFom		84	317	62.9	19.8	141	412
Lignin (ADL)		21	63	10.8	17.2	36	83
Gasbildung	ml/200mg TM	54	41.6	5.8	14.0	29.7	51.3
ELOS	g/kg TM	82	597	64.7	10.8	459	741
VQOS	%	89	67.5	7.0	10.4	53.5	82.8
ME	MJ/kg TM	89	9.21	0.9	9.3	7.5	11.3

[1)] Hinsichtlich der Silagen mit Ausnahme der Gasbildung korrigiert um den Verlust an flüchtigen Substanzen

Das Datenmaterial weist mit Variationskoeffizienten zwischen 20 und 24 % für die Faserfraktionen, 18 % für Rohprotein, 34 % für Rohfett sowie von etwa 10 % für die VQOS und den ME-Gehalt eine ausreichende Streuung zur Ableitung von Regressionsgleichungen auf. Der Ligningehalt (als Säure-Detergenzien-Lignin [ADL] bestimmt) war nur in einem Teil der Datensätze angegeben. Deshalb wurde diese Fraktion bei den Regressionsberechnungen nicht berücksichtigt. Die Werte sind jedoch zur Ergänzung und als Grundlage für zukünftige Aktualisierungen in die Tab. 2 aufgenommen. Auch die Werte für die Rohfasergehalte sind zur Charakterisierung des Materials genannt. Sie werden zur Berechnung der Referenzwerte für die ME benötigt. Die Rohfaser wurde jedoch nicht als Variable bei der Ableitung der Schätzgleichungen berücksichtigt. Die Ableitung der Schätzgleichungen erfolgte auf der Basis der ME-Gehalte sowie der Nährstoffgehalte und der enzymlöslichen Organischen Substanz (ELOS) sowie der Gasbildung (Gb) in der Organischen Substanz. Die Validierung sowie die Angabe des Standardfehlers und Bias (systematischer Fehler) erfolgten aber nach Umrechnung der Werte auf den ME-Gehalt in der Trockenmasse (TM).

In einem ersten Schritt wurden verschiedene Schätzgleichungen zur Vorhersage des Gehaltes an ME abgeleitet und anhand ihres Bestimmtheitsmaßes (B), der Reststreuung (sR) und des Bias miteinander verglichen. Zunächst wurde versucht, lediglich eine einzige Schätzgleichung mit Gültigkeit für das gesamte Material der Grobfutterleguminosen abzuleiten. Dies führte nicht bei allen Konservierungstypen und Aufwuchsnummern zu befriedigenden Ergebnissen. Daher wurde, ähnlich früheren Vorgehensweisen für Grasprodukte (GfE 1998), auch die getrennte Ableitung von Schätzgleichungen nach 1. Aufwuchs und Folgeaufwüchsen geprüft. In einem dritten Schritt wurden die abgeleiteten Schätzgleichungen am gesamten Datenpool jeweils getrennt nach Konservierungstyp sowie Aufwuchsnummer validiert. Die Gleichungen wurden mithilfe des Statistik-Datenpaketes SAS® unter Nutzung der Prozedur PROC REG bei schrittweiser Parameterauswahl abgeleitet. Berücksichtigt wurden bei Verwendung eines konstanten additiven Wertes folgende Variablen: Rohprotein,

Rohfett, ADFom bzw. aNDFom sowie ELOS und Gb, jeweils bezogen auf den Gehalt in der Organischen Substanz. Konzeptionell wurden erst Varianten unter Verwendung einer der beiden in vitro-Kenngrößen gerechnet, anschließend wegen der relativ geringen Korrelation zwischen ELOS und Gb (r = 0,64) auch beide gemeinsam in einer Ableitung genutzt. Es wurden schließlich nur die Variablen berücksichtigt, deren Signifikanzniveau $p < 0,15$ war.

3 Ergebnisse:

3.1 Regressionsgleichungen zur Berechnung der ME

Die Ergebnisse der Auswertung sind in Tabelle 3 dargestellt. Die Einbeziehung der aNDFom erwies sich in keinem Falle als sinnvoll, da das Signifikanzniveau des Regressionskoeffizienten jeweils deutlich geringer als das der ADFom war. Die Gleichungen 1 - 3 sind jeweils unter Einbeziehung des gesamten Datenpools ohne spezielle Berücksichtigung der Aufwuchsnummer abgeleitet worden. Diese Gleichungen sind im Hinblick auf Schätzgenauigkeit (B, s_R) nur bedingt miteinander vergleichbar, da sie jeweils auf unterschiedlich großen Datenpools beruhen. Trotz des vergleichsweise hohen Bestimmtheitsmaßes von B = 0,84 und der geringen Reststreuung s_R von 0,47 bzw. 0,50 MJ/kg OS wird die Sicherheit bei der Schätzung des ME-Gehaltes der Grobfutterleguminosen durch die gemeinsame Verwendung von ELOS und Gb noch verbessert. Ähnliches gilt für die getrennte Schätzung des ME-Gehaltes für den 1. Aufwuchs und die Folgeaufwüchse bzw. die Kategorie ohne Angabe der Aufwuchsnummer. Die Materialien, für die eine Aufwuchsnummer nicht bekannt war, wurden in der Auswertung zu den „Folgeaufwüchsen“ und nicht zum 1. Aufwuchs gezählt.

Tabelle 3: Bestimmtheitsmaß (B) und Reststreuung (s_R) der abgeleiteten Schätzgleichungen für den Gehalt an ME in der Organischen Substanz

Gleichung Nr.	n	Variablen	Aufwuchsnummer	B	s_R
ME_1	81	ADFom, XP, XL, ELOS	nicht differenziert	0.84	0.47
ME_2	54	ADFom, XP, XL, Gasbildung	nicht differenziert	0.84	0.50
ME_3	53	ADFom, XP, XL, ELOS, Gasbildung	nicht differenziert	0.89	0.43
ME_1_1	22	ADFom, XP, XL, ELOS	1	0.90	0.36
ME_2_1	17	ADFom, XP, XL, Gasbildung	1	0.89	0.42
ME_3_1	16	ADFom, XP, XL, ELOS, Gasbildung	1	0.89	0.36
ME_1_2	58	ADFom, XP, XL, ELOS	Folge und ohne	0.85	0.46
ME_2_2	36	ADFom, XP, XL, Gasbildung	Folge und ohne	0.86	0.50
ME_3_2	36	ADFom, XP, XL, ELOS, Gasbildung	Folge und ohne	0.91	0.41

3.1.1 Validierung der Gleichungen zur Schätzung der ME

Die Validierung erfolgte am Gesamtdatenpool, für die Kategorien Grünfutter, Silagen und Heu sowie getrennt nach 1. und Folgeaufwuchs bzw. Datenmaterial ohne Angabe der Aufwuchsnummer (Tabelle 4) mit Hilfe des Verfahrens „Leave-One-Out-Kreuzvalidierung“. Eine unabhängige Validierung war aufgrund der begrenzten Probenanzahl nach der Differenzierung in die verschiedenen Kategorien nicht möglich. Es zeigte sich, dass die Wahl der *in vitro*-Kennzahl in Bezug auf die Genauigkeit der Schätzung unerheblich ist, da der Standardfehler etwa gleich bleibt. Bei Vorhandensein von Analysenergebnissen zu beiden in vitro-Kennzahlen lässt sich die Schätzgenauigkeit noch einmal verbessern. Hinsichtlich des Konservierungstyps ist die Robustheit einer allgemeinen Gleichung, die dies nicht berücksichtigt, nur für die Verwendung von ELOS gegeben. Bei Nutzung der Gb für den – allerdings nicht deckungsgleichen – Datenpool ergibt sich für das Grünfutter eine deutliche Unterschätzung bei Anwendung der Schätzgleichung. Markanter jedoch ist die recht deutliche und generelle Unterschätzung des ME-Gehaltes für den 1. Aufwuchs um mehr als 0,2 MJ ME/kg TM bei Verwendung der Gleichungen 1 - 3. Es bestehen somit Unterschiede zwischen den Pflanzen aus dem 1. Aufwuchs und den Folgeaufwüchsen eines Jahres, die durch die üblichen Untersuchungskennzahlen, auch unter Einbeziehung der beiden in vitro-Kennzahlen für die Verdaulichkeit, nicht abgebildet wird. Bei getrennter Ableitung von Schätzgleichungen für Materialien aus dem 1. Aufwuchs und den Folgeaufwüchsen wird diese gerichtete Abweichung weitgehend aufgehoben. Es bleibt jedoch auch in diesem Fall eine gerichtete Unterschätzung des ME-Gehaltes des Grünfutters bei Verwendung der Gb.

Tabelle 4: Standardfehler (s%) und Bias bei der Validierung der abgeleiteten Schätzgleichungen zur Berechnung des ME-Gehaltes (MJ ME/kg TM) differenziert nach Konservierungsart und Aufwuchsnummer

Gleichung	Parameter	alle	Konservierungstyp			Aufwuchsnummer		
			Frisch	Siliert	Getrocknet	1	Folge-aufwüchse	Ohne Angabe
ME_1	n	81	24	34	23	23	42	16
	Mittelwert	9.22	9.78	9.12	8.78	9.55	9.22	8.73
	% Standardfehler	4.4	5.2	4.3	3.3	3.5	4.4	3.7
	Bias	0.00	-0.04	-0.04	0.11	-0.25	0.05	0.24
ME_2	n	54	13	19	22	18	24	12
	Mittelwert	9.11	9.90	8.85	8.88	9.41	9.06	8.78
	% Standardfehler	4.7	2.5	5.3	4.6	3.7	4.4	5.4
	Bias	0.00	-0.28	0.03	0.14	-0.23	0.06	0.23
ME_3	n	53	13	19	21	17	24	12
	Mittelwert	9.11	9.97	8.86	8.80	9.45	9.07	8.71
	% Standardfehler	4.0	3.4	4.6	3.4	3.6	4.0	3.2
	Bias	0.00	-0.20	0.04	0.09	-0.20	0.06	0.16
ME_1_1/1_2	n	81	24	34	23	23	42	16
	Mittelwert	9.21	9.77	9.16	8.76	9.80	9.13	8.63
	% Standardfehler	4.3	5.0	4.0	3.9	3.6	4.6	4.3
	Bias	0.01	-0.03	-0.02	0.08	0.01	-0.05	0.14
ME_2_1/2_2	n	54	13	19	22	18	24	12
	Mittelwert	9.15	9.92	8.94	8.92	9.70	8.97	8.70
	% Standardfehler	5.6	2.7	7.0	4.9	6.1	47.6	6.7
	Bias	0.04	-0.26	0.07	0.18	0.01	-0.03	0.16
ME_3_1/3_2	n	53	12	20	21	17	24	12
	Mittelwert	9.11	9.97	8.93	8.78	9.63	8.96	8.65
	% Standardfehler	4.5	4.0	5.1	3.9	4.8	4.3	4.3
	Bias	0.00	-0.21	0.05	0.07	-0.01	-0.04	0.10

Abbildung 1 zeigt die Differenzen der nach Gleichung 1 geschätzten ME-Gehalte von den aus den verdaulichen Rohnährstoffen (Verdaulichkeitsstudien mit Schafen) berechneten Werten. Über fast die gesamte Variationsbreite schätzt Gleichung 1 den ME-Gehalt mit etwa gleicher Reststreuung. Abbildung 2 zeigt dagegen deutlich die negativen Abweichungen des über Gleichung 1 geschätzten ME-Gehaltes von Materialien des 1. Aufwuchses. Diese Unterschätzung des ME-Gehaltes wird in Gleichung 1 durch die tendenzielle Überschätzung der Materialien ohne Angabe der Aufwuchsnummer ausgeglichen. Bei getrennter Auswertung des 1. Aufwuchses ist diese Fehleinschätzung für den 1. Aufwuchs und die Materialien ohne Angabe der Aufwuchsnummer nicht mehr vorhanden (Abbildung 3).

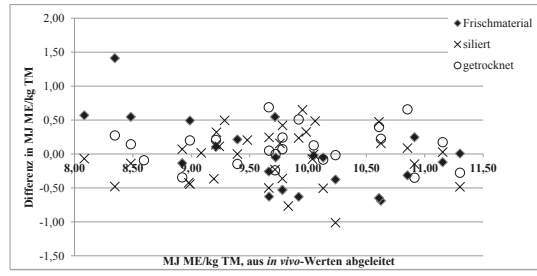

Abbildung 1: Differenzen der Schätzwerte für die Gehalte an ME zu den in Verdaulichkeitsstudien mit Hammeln ermittelten ME-Gehalten nach Konservierungsart (Gleichung ME_1)

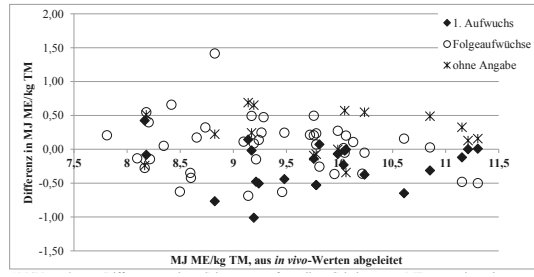

Abbildung 2: Differenzen der Schwarzwerte für die Gehalte an ME zu den in Verdaulichkeitsstudien mit Hammeln ermittelten ME-Gehalten nach Aufwuchsnummer (Gleichung ME_1)

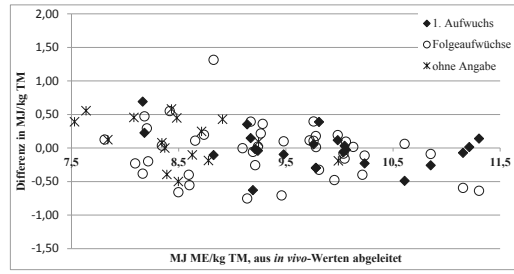

Abbildung 3: Differenzen der Schätzwerte für die Gehalte an ME zu den in Verdaulichkeitsstudien mit Hammeln ermittelten ME-Gehalten nach Aufwuchsnummer (Gleichungen ME_1_1 und ME_1_2)

3.1.2 Empfohlene Gleichungen zur Schätzung der ME von Grobfutterleguminosen

In Anlehnung an das Vorgehen bei Gras- und Maisprodukten (GfE 2008) und Mischfuttermitteln für Wiederkäuer (GfE 2009) empfiehlt der Ausschuss für Bedarfsnormen (AfBN) zur Schätzung der ME in Grobfutterleguminosen jeweils zwei alternative Gleichungen unter Berücksichtigung von entweder ELOS oder Gb (Tab. 5). In beiden Varianten ist jedoch für den 1. Aufwuchs eine separate Gleichung anzuwenden, weil die Genauigkeit der Schätzung hierdurch höher ist. Für Folgeaufwüchse wird dementsprechend eine andere Gleichung empfohlen. Liegen keine Angaben zur Aufwuchsnummer vor, wird die Gleichung für Folgeaufwüchse genutzt. Nicht gekennzeichnete Proben des 1. Aufwuchses werden dann um etwa 0,3 MJ ME/kg TM unterbewertet. Zur Vermeidung dieses systematischen Fehlers ist es für den Routinebetrieb daher wichtig, dass die Probeneinsender eine eindeutige Angabe zur Aufwuchsnummer der Proben machen.

Tabelle 5: Empfohlene Gleichungen zur Schätzung des ME-Gehaltes in der Organischen Substanz von Grobfutterleguminosen

Basierend auf ELOS:					
Erster Aufwuchs			Folgeaufwüchse bzw. ohne Aufwuchsangabe		
ME =	11.91		ME =	9.83	
	- 0.01034	• ADFom		- 0.01010	• ADFom
	+ 0.00389	• XP		+ 0.00039	• XP
	+ 0.01870	• XL		+ 0.00802	• XL
	+ 0.00191	• ELOS		+ 0.00571	• ELOS
B =	0.904		B =	0.852	
s_R =	0.36		s_R =	0.46	
ME in MJ/kg OS; XP, XL, ADFom, ELOS in g/kg OS					

Basierend auf Gasbildung:					
Erster Aufwuchs			Folgeaufwüchse bzw. ohne Aufwuchsangabe		
ME =	12.49		ME =	11.09	
	- 0.01140	• ADFom		- 0.01040	• ADFom
	+ 0.00425	• XP		+ 0.00497	• XP
	+ 0.02690	• XL		+ 0.00750	• XL
	+ 0.01683	• Gb		+ 0.0351	• Gb
R^2 =	0.891		R^2 =	0.862	
s_R =	0.42		s_R =	0.50	
ME in MJ/kg OS; XP, XL, ADFom in g/kg OS; Gb in ml/200 mg OS					

Die Umrechnung auf den ME-Gehalt in der Trockenmasse erfolgt dann für die konkret zu bewertende Probe anhand ihres Gehaltes an Organischer Substanz nach folgender Gleichung:

ME (MJ/kg TM) = ME (MJ/kg OS) • [1000 – XA (g/kg TM)]/1000

Für Milchkühe kann die Nettoenergie-Laktation (NEL) aus der ME unter Berücksichtigung der Umsetzbarkeit der Bruttoenergie (GE) (q) gemäß GfE (2001) wie folgt errechnet werden:
NEL (MJ) = 0,6 [1 + 0,004 (q – 57)] ME (MJ), wobei q = ME/GE • 100

Dafür muss der Gehalt an GE, falls dieser nicht bombenkalorimetrisch gemessen wurde, zunächst nach folgender Gleichung berechnet werden:

GE (MJ/kg TM) = 0,0239 • XP (g/kg TM) + 0,0398 • XL (g/kg TM) + 0,0201 • XF (g/kg TM + 0,0175 • NfE (g/kg TM)

Für diese Berechnung muss der Gehalt an Rohfaser und N-freien Extraktstoffen (NfE) bekannt sein. Falls diese Werte nicht bekannt sind, kann die Umrechnung der ME auf den Gehalt an NEL vereinfacht nach folgender Gleichung erfolgen (Weißbach et al. 1996):

NEL (MJ) = ME [0,46 + 12,38 • ME / (1000 – XA)], wobei: ME in MJ und XA in g/kg TM

3.2 Regressionsgleichungen zur Schätzung der VQOS

Da die Verdaulichkeit eine Voraussetzung für die Berechnung der Energiewerte, sie aber unabhängig von der Ausgestaltung eines Energiebewertungssystems ist, wurden auch Gleichungen zur Schätzung der VQOS abgeleitet. Zur Ableitung der Schätzgleichungen für die VQOS wurden ebenfalls die Konzentrationen an Rohprotein, Rohfett, ADFom, aNDFom sowie mindestens ein in vitro-Kriterium verwendet. Die Berechnungen

erfolgten auf der Basis der Gehalte in der Organischen Substanz. Parameter, deren Regressionskoeffizient nicht mindestens ein Signifikanzniveau von p=0,15 erreichten, wurden als nicht signifikant ausgeschlossen. Ähnlich dem Vorgehen bei der Schätzung der ME wurde im ersten Schritt eine allgemeingültige Gleichung für alle Konservierungstypen und Aufwuchsangaben abgeleitet. Im zweiten Schritt wurde für die Proben des 1. Aufwuchses eine eigene Gleichung abgeleitet. Die Ergebnisse der Regressionsanalysen sind in Tab. 6 dargestellt.

Tabelle 6: Bestimmtheitsmaß (B) und Reststreuung (s_R) der abgeleiteten Schätzgleichungen für die Berechnung der Verdaulichkeit der Organischen Substanz (in %)

Gleichungs-Nr.	n	Variablen	Aufwuchsangaben	B	s_R
VQOS_1	81	ADFom, CP, ESOM	nicht differenziert	0.82	3.17
VQOS_2	54	ADFom, GP	nicht differenziert	0.82	3.37
VQOS_3	53	ADFom, ESOM, GP	nicht differenziert	0.87	2.93
VQOS_1_1	22	ADFom, ESOM	1	0.89	2.31
VQOS_2_1	17	ADFom, GP	1	0.88	2.76
VQOS_3_1	16	ADFom, ESOM, GP	1	0.89	2.72
VQOS_1_2	58	ADFom ESOM	Folge und ohne	0.81	3.23
VQOS_2_2	36	ADFom, GP	Folge und ohne	0.83	3.36
VQOS_3_2	36	ADFom, ESOM, GP	Folge und ohne	0.88	2.91

Auffallend ist die deutliche Reduzierung der Anzahl an unabhängigen Parametern gegenüber der Schätzung des ME-Gehalts (Tab. 3), womit eine größere Wichtung der in vitro-Parameter für die Schätzung der VQOS im Vergleich zur ME-Schätzung einhergeht. Durch die Nutzung beider in vitro-Kenngrößen konnte eine gewisse Verbesserung der Schätzgenauigkeit gegenüber der alternativen Nutzung nur einer einzelnen Kenngröße erreicht werden.

3.2.1 Validierung der Gleichungen zur Schätzung der VQOS

Die Validierung erfolgte wiederum am Gesamt-Datenpool sowie für die Teilkategorien (Tab. 7) mit Hilfe des Verfahrens „Leave-One-Out-Kreuzvalidierung“. Ähnlich der Vorgehensweise bei der Schätzung der ME ergeben sich für die Schätzung der VQOS bei Nutzung nur jeweils einer Gleichung über alle Aufwuchsbedingungen und Konservierungstypen grundsätzlich gerichtete negative Abweichungen für Proben des 1. Aufwuchses von 1,5 bis 1,8 Prozentpunkten und positive Abweichungen vor allem bei den nicht durch Aufwuchsangaben gekennzeichneten Materialien. Hinzu kommen Ungenauigkeiten bei der Differenzierung der VQOS von frischem und trockenem Erntematerial bei Verwendung der Gb (Gleichung VQOS_2). Bei getrennter Ableitung von Schätzgleichungen für Materialien aus dem 1. Aufwuchs und den Folgeaufwüchsen ist eine gerichtete Abweichung nicht vorhanden (Gleichung VQOS_1_1+2 bis VQOS_3_1+2; Abbildung 4). Es bleibt jedoch der Mangel der gerichteten Unterschätzung von Grünfutter bei Verwendung der Gb.

Tabelle 7: Standardfehler und Bias bei Anwendung der abgeleiteten Gleichungen zur Schätzung der VQOS (in %) am Gesamtdatenmaterial sowie an Teilkategorien

Gleichung	Parameter	alle	Konservierungstyp			Aufwuchsnummer		
			Frisch	Siliert	Getrocknet	1	Folge-aufwüchse	Ohne Angabe
VQOS_1	n	81	24	34	23	23	42	16
	Mittelwert	67.7	72.0	66.1	65.6	69.3	68.6	63.2
	% Standardfehler	4.6	5.6	4.2	3.7	3.4	4.7	4.5
	Bias	-0.01	-0.53	0.11	0.37	-1.68	0.35	1.44
VQOS_2	n	54	13	19	22	18	24	12
	Mittelwert	67.3	74.4	63.4	67.1	68.4	68.4	63.6
	% Standardfehler	4.9	3.0	5.3	4.8	4.0	4.5	5.8
	Bias	0.00	-2.29	0.07	1.18	-1.69	0.48	1.58
VQOS_3	n	53	13	19	21	17	24	12
	Mittelwert	67.3	74.6	63.7	66.0	68.6	68.4	63.2
	% Standardfehler	4.2	3.7	4.9	3.5	3.5	4.0	4.7
	Bias	0.01	-1.73	0.80	0.36	-1.47	0.46	1.17
VQOS_1_1/ VQOS_1_2	n	81	23	35	23	23	42	16
	Mittelwert	67.7	72.1	66.3	65.4	71.0	67.8	62.6
	% Standardfehler	4.7	5.5	4.4	4.2	3.6	4.9	5.5
	Bias	-0.02	-0.50	0.20	0.1	0.04	-0.39	0.83
VQOS_2_1/ VQOS_2_2	n	54	12	20	22	18	24	12
	Mittelwert	67.4	74.9	63.5	66.7	70.0	67.6	62.9
	% Standardfehler	5.0	3.6	5.1	5.4	4.4	4.9	6.2
	Bias	-0.02	-1.77	0.15	0.88	-0.06	-0.34	0.85
VQOS_3_1/ VQOS_3_2	n	53	12	20	21	17	24	12
	Mittelwert	67.1	75.0	63.7	65.8	69.6	67.6	62.4
	% Standardfehler	4.4	4.1	4.3	4.4	3.7	4.4	5.4
	Bias	-0.20	-1.72	0.31	0.18	-0.46	-0.32	0.4

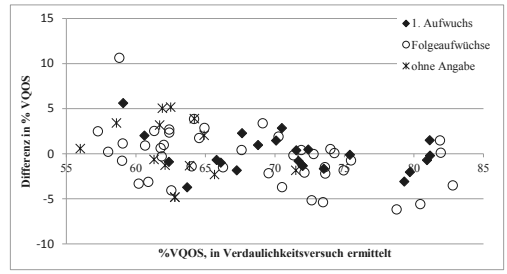

Abbildung 4: Differenzen der Schätzwerte für die Verdaulichkeit der Organischen Substanz (VQOS) auf Basis von ELOS im Vergleich zu den an Schafen ermittelten Werten nach Aufwuchsnummer (Gleichung VQOS_1_1 und VQOS_1_2)

3.2.2 Empfohlene Gleichungen zur Schätzung der VQOS von Grobfutterleguminosen

Der AfBN empfiehlt auch für die Schätzung der VQOS von Grobfutterleguminosen zwei alternative Gleichungen unter Verwendung der ELOS sowie der Gb mit Differenzierung nach Aufwuchsnummer (Tab. 8). In beiden alternativen Empfehlungen ist für den 1. Aufwuchs eine eigene Gleichung anzuwenden. Für Folgeaufwüchse ist die entsprechende Gleichung zu verwenden. Werden keine Angaben zur Aufwuchsnummer gemacht, ist die Gleichung für die Folgeaufwüchse zu nutzen. Die Anwendung der zweiten Gleichung auf nicht gekennzeichnete 1. Aufwüchse führt zu einer Unterschätzung der Verdaulichkeit von etwa 2,5 Prozentpunkten.

Tabelle 8: Empfohlene Gleichungen zur Schätzung der VQOS in Grobfutterleguminosen

Basierend auf ELOS					
Erster Aufwuchs			Folgeaufwüchse bzw. ohne Aufwuchsangabe		
VQOS =	81.71		VQOS =	70.77	
	- 0.0711	• ADFom		- 0.0683	• ADFom
	+ 0.0195	• ELOS		+ 0.0302	• ELOS
B =	0.892		B =	0.806	
s_R =	2.31		s_R =	3.23	
VQOS in %; ADFom, ELOS in g/kg OS					

Basierend auf Gasbildung					
Erster Aufwuchs			Folgeaufwüchse bzw. ohne Aufwuchsangabe		
VQOS =	95.72		VQOS =	77.90	
	- 0.0859	• ADFom		- 0.0711	• ADFom
	+ 0.0964	• Gb		0.2997	• Gb
B =	0.859		B =	0.832	
s_R =	2.813		s_R =	3.36	
VQOS in %; ADFom in g/kg OS; Gb in ml/200 mg OS					

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Statement on energy evaluation of feeds for pigs based on metabolisable energy versus net energy

Stellungnahme zur energetischen Futterbewertung beim Schwein auf Basis Umsetzbarer Energie versus Nettoenergie

Summary

This statement is a comprehensive presentation of the scientific arguments and aspects relevant to practical feeding and formulation of rations and compound feeds which are important in evaluating feeds for pigs on the basis of metabolisable energy (ME) and net energy (NE).

The ME indicates the capacity of a feed to provide energy for the animal's metabolism. The NE additionally includes the nutrient-specific heat losses occurring in the metabolism and represents the retained energy (RE) in the growing animal. However, because the extent of these losses is not constant, especially for protein, and the energy requirements for basal metabolism, gestation, thermoregulation and physical activity must be expressed as equivalents to RE, this leads to problems or inaccuracies. The RE as part of performance prediction is therefore only partly successful with a NE system. Only by knowing the composition of the ration, especially its protein and amino acid contents, and the animal's characteristics, notably protein deposition capacity, is it possible to predict growth, body composition and nutrient excretion.

Extensive calculations carried out in cooperation with a large feed manufacturer have shown that optimisation of compound feeds based on NE versus ME does not lead to a reduction in cost or to a decrease in protein concentration of the feed. Any specific NE system also has the disadvantage of incompatibility with other NE systems, whereas the NE values of different NE systems can generally be calculated from the ME value. A major problem of using NE values is that they cannot be verified experimentally due to the great effort associated with respiration studies. In contrast, ME values can be determined using digestibility studies complemented by urine collection. New findings on nutrient utilisation efficiency and energy requirements can be inserted into recommendations for energy supply more easily and more quickly in a ME system than in NE systems, because in the latter the feed values and hence the feed tables would need to be amended.

It is therefore not advisable, either from a scientific point of view or in the interests of agricultural practice, to convert from ME to one of the existing NE systems. According to current knowledge, ration formulation based on ME is to be regarded as best practice.

1. Introduction

Precise determination of the energy supply capacity of feeds is crucial because the economic value of most feeds depends largely on the energy content and animal performance is strongly influenced by the energy supply. In feeds with low fat content, it is mainly the fat content and the digestibility of the fibre fractions which determine the variation in energy feed value, since proteins, starch and sugars generally have high precaecal digestibility (pcd). Losses occurring during fibre fermentation (in the form of methane, fermentation heat and microbial matter) also reduce the energy value of fibre-rich feeds. Menke (1987) postulated that the task of energy evaluation of feeds is to estimate the losses mainly caused by characteristics of the feed. According to this postulate, losses via methane production and urinary excretion must be taken into account in addition to losses via faecal excretion (metabolisable energy, ME). If the metabolic heat produced due to nutrient supply is also included, this leads to net energy (NE). The purpose of this statement is to present the state of the art with regard to energy evaluation of feeds for pigs in Germany and to identify the reasons which led to the decision to carry out evaluation at ME level. Possible benefits and limitations of evaluation at NE level are also presented, together with associated drawbacks. This statement is an extension of the arguments previously published by the Committee of Nutrient Requirement Standards (Ausschuss für Bedarfsnormen, AfBN) of the Society of Nutrition Physiology (Gesellschaft für Ernährungsphysiologie, GfE) in December

2015 in the form of a press release[1]. The text is based on statements by the GfE (2008, chapter 2) and on publications by Susenbeth (2005, 2010 and 2016) which deal with some aspects in more detail.

2. Evaluation at the level of metabolisable energy

The guiding principle adopted by the AfBN was to define energy feed value as the potential to provide energy for the animal's metabolism, and to calculate it from the concentrations of digestible nutrients (GfE, 2008). This necessarily leads to evaluation at ME level. However, predicting performance and hence retained energy (RE) cannot be a primary task of energy evaluation of feeds. This is because energy utilisation is also affected by amino acid supply, performance potential and the type of performance of the animals. As a result, performance can be predicted only to a very limited extent based solely on the characteristics of a feed (see the comments below).

The equation for calculating the ME content is (GfE, 2008):

ME = 20.5 DXP + 39.8 DXL + 17.3 ST + 16.0 SU + 14.7 DOR (kJ or g/kg dry matter [DM]),

where DXP stands for digestible crude protein, DXL digestible crude lipids, ST starch, SU sugar, DOR digestible organic residue (DOR = DOM - DXP - DXL - ST - SU; DOM = digestible organic matter). This formula is based on extensive studies conducted by the Rostock research group (Jentsch et al., 2001). Only the factor for DOR has been modified compared with the original equation. This takes account of methane losses and fermentation heat. This equation is very robust and reliable in its application since the factors are almost identical to the theoretical values. The factor 20.5 for DXP corresponds to a ME value for protein where protein is utilised efficiently in the metabolism, i.e. urinary N excretion is low. Of note, the factors for ST and SU refer not to the digestible fractions but to the total content in the feed; however, due to their generally very high pcd, these factors are almost identical to the gross energy value.

The inclusion of DOR, whereby the fibre fraction and other non-starch polysaccharides (NSP) are determined not by analysis but by calculation, leads to a substantial advantage in terms of reliability when applying the equation[2]. Analytical errors, especially in the case of carbohydrate fractions, have only a marginal effect on the calculated ME value since any over- or under-estimation of nutrient levels is quantitatively offset by DOR. The factors for the three fractions DOR, ST and SU do not differ considerably, so the effect of an analytical error on the ME value of the feed is marginal. For example, if the starch content is under-analysed by 5 percentage points, the DOR content increases by 5 percentage points. Despite this substantial analytical error, the resulting error for determination of the ME content of the feed is only (17.3 kJ/g - 14.7 kJ/g) • 50 g = 0.13 MJ/kg DM. The GfE (2008) points out that applying the formula to feed materials containing substantial levels of pectins or alcohols, for example, can lead to false estimations. In these cases the accuracy of calculation can be improved if the quantity and energy value of such nutrients are taken into account separately and the nutrient fraction concerned is reduced accordingly if necessary[3]. The same applies to feeds in which the starch has a low pcd; post-ileally digested starch is attributed to DOR.

The question remains of whether all losses associated with fermentation are covered to the necessary extent by using a factor of 14.7 for DOR, which at first appears fairly high and is only 2.6 units lower than that for starch. These 2.6 kJ/g only cover the losses via fermentation heat and methane production. The microbial mass produced during fermentation is another, greater source of loss and amounts to more than 20 % of the fermented energy. However, this loss is not covered by the factor for the energy value of DOR, because in digestibility studies the microbial matter is contained in the crude protein fraction as well as in other fractions, and hence it is considered via faeces analysis. It would therefore be incorrect to conclude from the formula that 1 g of fermented fibre provides 14.7 kJ ME; rather, according to this formula too, the energy value is below 11.2 kJ ME/g if all fermentation-related losses, including microbial matter, are covered solely by the factor for DOR.

The ME equation of GfE (2008) can be used very successfully to describe the energy provided for the metabolism by the feed. There is a slight limitation due to the variability of urinary energy loss. However, the level of urinary energy losses cannot be attributed to the feed materials because these losses are mainly a result of protein content and quality in the total ration, which is determined by the pcd and pattern of the

1 http://www.gfe-frankfurt.de/download/GfE_Nettoenergie_Schwein.pdf.

2 An organic residual fraction is also included in other authors' equations (Susenbeth, 2005). Note, however, that this fraction is defined or calculated differently in the respective equations.

3 This procedure is followed by the CVB (2016) in Formula F.V09.

amino acids, as well as by the animals' protein deposition.[4]

3. Performance prediction based on metabolisable energy

Energy evaluation of feeds cannot be carried out separately from determining requirements or predicting performance (Menke, 1987). It is not the task of an energy evaluation to predict performance. However, energy feed value must be defined in such a way that it can be used as one of the determinants for performance prediction. For example, the utilisation factors (k = RE/ME) obtained by Jentsch et al. (2000a) and confirmed by comprehensive experiments can be used to predict energy retention (RE) during growth. For starch, protein and fat, the factors are 0.757, 0.623 and 0.859 respectively. If starch equals 100 %, protein and fat are 82 % and 113 % respectively[5]. To simplify in practice, however, a mean k_{pf} value of 0.72 to 0.74 and a kl value of 0.72 (Susenbeth, 19966; GfE, 2008) can be used, based on the ME of the ration, to predict the energy retained in the body or transferred into milk; here, differences between nutrients in terms of utilisation efficiency are not considered.[7,8] This means that predicting RE based solely on ME, without knowing the respective nutrient levels, necessarily entails a degree of inaccuracy. This is the crucial fact which is used as an argument to support the necessity of feed evaluation at NE level. However, this inaccuracy (constant k value, no consideration of nutrient differences) is very small for a situation where protein supply meets requirements. The same is true for fat: energy utilisation increases by only 0.001 per percentage point of fat in the ration.

Assuming constant[9] ME utilisation independent of nutrient composition, the accuracy of the RE calculation actually decreases slightly only for rations with higher fat contents and excess protein levels, and NE might have an advantage in this case. For a more precise estimation of RE, which also requires knowledge of the animal's protein deposition and the content and quality of protein in the ration and also takes account of housing conditions if necessary, evaluation of the energy supplied by the feed on the basis of ME is not an obstacle; rather, it can and should be done on this basis. For the reasons mentioned before, the argument that a more accurate estimation of energy retention can be obtained with NE is irrelevant for the purposes of practical ration formulation and feeding.

4. Energy evaluation at the level of net energy

The central concern of energy evaluation of feeds at NE level is, as mentioned above, the prediction of RE while considering the different *energy utilisation of the individual nutrients*. A critical assessment of NE therefore has to be done firstly with this objective in mind:

1. The NE indicates the RE (mainly as fat deposition), which is calculated from the ME content of the individual nutrients and their utilisation factors. However, the RE value derived in this way is associated with a fundamental problem which is especially apparent in the case of protein. It must be assumed that the **utilisation efficiency of ME from protein** is not constant and is different for protein deposition than for pure energy use. The energy value of protein can therefore be influenced by factors which are not caused by characteristics of the feed and hence are unknown in the evaluation process. Only by knowing the total ration, protein quality and animal's performance is it possible to predict the protein deposition and thus the efficiency of protein utilisation. For example, the Dutch institute Schothorst Feed Research (2016) reiterates that the main limitation of NE systems is that post-absorptive nutrient use cannot be considered and that consequently the use of a uniform energy value for protein does not take account of its different potential

4 This problem of variability in urinary energy excretion applies equally to NE. A way to improve the accuracy of the ME value (and hence of the NE value) of a ration and avoid this uncertainty is to include the animal's protein utilisation, which is the main cause of differences in urinary energy excretion. A proposal for such a calculation has been worked out (A. Susenbeth, unpublished) in which two energy values are used for protein: a higher value for protein which is used for protein deposition and has the maximum protein utilisation of the ideal protein, and a much lower value for protein which exceeds the minimum requirements (i.e. the minimum supply of precaecally digestible crude protein; see Table 4.9; GfE, 2008) and where the corresponding nitrogen is completely excreted via the urine.

5 The use of the utilisation coefficients given by Noblet (2006) leads to divergent results, as these coefficients differ considerably from those of Jentsch et al. (2000a).

6 The higher k_{pf} value given here compared with the original publication results from the use of the energy values of 23.8 kJ/g crude protein and 38.7 kJ/g crude lipids.

7 The k_p value (= 0.56) and k_f value (= 0.74) (GfE, 2008) must be differentiated from the different energy utilisation of the individual nutrients. These values indicate the efficiency of ME utilisation for energy retention as protein or fat, irrespective of the nutrient concentration of the ration, i.e. they focus not on the origin of the energy but on its target, and therefore apply to rations which have common levels of protein, fibre and lipids.

8 A k_{pf} value of 0.73 to 0.74 is also obtained by INRA (2004; equation NE7) for an average ration composition.

9 See footnote 7.

uses. Indeed, Boisen (2007), who developed the Danish system of Potential Physiological Energy, concludes that NE is not a suitable basis for feed evaluation. He has considered the criticism raised previously by other authors, namely that NE applies only to a specific production and that the animal's response in the form of RE is also inadequate as a precise measure of feed energy value because influences stemming from the animal itself lead to variability in such a feed energy value.

The inability to define a generally valid energy value for protein due to this variability in protein utilisation supports evaluation at ME level, since this problem does not need to be considered - or can be left open - in feed evaluation based on ME. Two major NE systems, the Rostock feed evaluation system (Beyer et al. 2003, based on Jentsch et al., 2001) and the French system (INRA, 2004, based on Noblet et al., 1994), obviously give the energy value of protein for sole energy use. In the future, if proven specific utilisation factors were available for the respective protein use (protein deposition or energy use), they could be included in NE systems only with difficulty because the feed evaluation might have to be changed and feed tables revised. In the case of ME, however, they would be relatively easy to include in determining requirements or predicting performance. Besides the variability in utilisation, there are additional conceptional problems with evaluation at NE level:

2. The established NE values are above all only valid for performance in the form of energy retention during growth. **Milk production** does show a similar efficiency of ME utilisation. However, in a NE system, a separate utilisation factor would have to be introduced for lactose production. The problem is even more apparent if **gestation** requirements have to be determined: Since ME utilisation for gestation is much lower than for growth, the gestation requirements - given as NE for growth - are several times greater than the actual energy retention in foetuses and adnexa. A formal conversion is possible, but difficult to communicate.

3. There is an even greater problem with regard to **maintenance** requirement, since this also has to be given as RE. After all, even in intensively growing animals, around one third of total energy requirement is allocated to maintenance. It is known not only that utilisation efficiency in maintenance differs from that in growth, but also that the ratios between utilisation factors for nutrients differ (Chudy and Schiemann, 1969; Blaxter, 1989; Jentsch et al., 2000a).

4. Requirements for **physical activity** and **thermoregulation** *can only* be given in ME, since the ME expended on them is wholly converted into heat and NE values for such requirements do not exist. The heat production is greater than the necessary requirements expressed as NE. Such requirements which are not allocated to maintenance and growth can amount to 15 % of total requirements under commercial housing conditions for growing pigs (Naatjes et al., 2014). It has also been observed that fibre-rich feeding reduces physical activity. This results in lower heat production caused by activity, compensating for the increased fermentation heat (Rijnen, 2003); as a result, the measured RE value does not correspond to the NE value of fibre. Such effects should be considered when calculating NE, since this otherwise leads to an undesirable mixing of requirement determination and feed evaluation.

5. There is a **methodological constrain with experimental determination of the NE value**. To determine the RE of a feed, respiratory measurements have to be carried out in studies where the feed is added to a basal diet. Because this is generally not done due to the high effort involved, an assumption is necessary for the level of basal metabolism10; this value is added to the measured RE value. This assumed value is not the same for the different NE systems and the actual basal metabolism can also be influenced by experimental conditions, especially the length of food withdrawal. This problem is therefore a crucial, fundamental criticism of NE systems. It also means that NE values differ between different NE systems; this poses major difficulties for conversion between NE systems. If new information on maintenance or basal metabolism becomes available, it could not be implemented into recommendations for supply of energy but would lead to modified NE values for feeds and hence to a revision of the feed tables.

6. **Experimental verification of NE values** is theoretically possible but unrealistic: on the one hand, because the value defined in each system for maintenance or basal metabolism has to be adopted, and represents therefore not a measured value; on the other hand, because only a few institutions currently have respiration chambers for large animals. Digestibility studies, which are sufficient for ME determination and can be complemented by urine collection, can be regarded as acceptable for the purposes of feed evaluation, whereas the total metabolism studies required for NE determination are not really feasible.

10 Basal metabolism is defined as heat production without food intake, and therefore corresponds to the body nutrients mobilised in response to hunger (negative RE). For this reason, no maintenance requirement for NE can be defined since the maintenance requirement corresponds to the ME supply required to prevent mobilisation of body nutrients. Maintenance requirement is therefore greater than basal metabolism.

5. Current net energy systems

An assessment of energy evaluation of feed according to NE must consider the differences between NE systems, in addition to the general aspects outlined above. Three current NE systems11 based on RE during growth implemented the following formulas:

Rostock system (Beyer et al., 2003)

NE = 11.0 DXP + 34.0 DXL + 12.7 ST + 11.6 SU + 12.0 DOR

INRA (2004)

NE = 12.1 DXP + 35.0 DXL + 14.3 ST + 11.9 SU + 8.6 DOR

CVB (2016)[12]

NE = 11.70 DXP + 35.74 DXL + 14.14 ST + 12.73 pd SU + 9.74 · fermented carbohydrates[13]

Key aspects of these equations are addressed below. The factor for DXP differs between the NE systems only to a small extent. This is the case also for DXL and for SU. The value given for DOR in the Rostock system applies to rations with an energy digestibility higher than 80 %; however, if the energy digestibility is only 60 or 65 % for example, the factor is 9.2 or 9.9 and is therefore close to the other systems. This also shows that the energy contributed by this fraction tends to be underestimated at least for normal energy-rich rations according to INRA (2004) and CVB (2016). Due to the high ST content of many feeds, however, the differences in the factor for ST are more important. Based on respiration studies measuring the effect of added pure starch on energy retention, NE values above 14 kJ per gram of starch are to be regarded as unrealistically high. Rather, the energy utilisation of starch is consistently in the range of 75 to 76%[14]. The common statement that protein is under-valued in the NE system according to INRA is therefore not correct. Rather, there is a substantial over-valuation of starch, which is associated with a relative decrease in the value of the other nutrients. Because the selection of individual components in compound feed formulation focuses not on the absolute energy values of the nutrients but on their relative values, starch-rich components are over-evaluated, especially compared to protein- and fibre-rich components.

6. Summarising facts

6.1. NE systems

The advantages and limitations of feed evaluation based on NE can be summarised as follows:

1. With NE systems, energy retention is more accurately predicted in rations with high protein contents (i.e. exceeding minimum requirements) and in fat-rich feeds.

2. The energy value of starch is over-estimated by the NE according to INRA (2004) and CVB (2016).

3. The requirements for maintenance, gestation, milk production, physical activity and thermoregulation must be converted into NE growth, which makes the system harder to understand and difficult to communicate.

4. The different NE systems provide different values for the same feed and are not compatible.

5. Experimental verification of a feed value is to be regarded as unrealistic due to the high effort involved.

6. If new findings on energy metabolism are taken into account in NE systems, the feed tables will have to be changed. This especially concerns the energy value of protein, which is to be regarded as variable, and the basal and maintenance requirements.

6.2. ME system

The advantages and limitations of feed evaluation based on ME can be summarised as follows:

1. The ME describes the energy provided for the metabolism. A slight limitation is due to the fact that the energy losses via urine also depend on the protein quality and the animal's protein deposition. Only a system

11 The 'Potential Physiological Energy' in the Danish system (Tybirk et al., 2006; Boisen, 2007) is derived from the ATP-producing capacity of the nutrients. The intention of this system agrees with that of ME as the energy supply capacity of the feed. The energy values of the respective nutrients are close to NE values but not identical to them, which is the reason why they are not listed here for comparison.

12 Formula V.F10; (Formula V.F09 also includes volatile substances and glycerol.)

13 Total of digestible NSP, fermented SU and fermented ST

14 Virtually identical values have been found in rats (75.5; Nehring et al., 1961) and in humans (75.8; Jentsch et al., 2000b).

based on digestible energy (DE) would be unaffected, as would every NE system. The ME therefore does not include differences in the energy utilisation of nutrients. The ME value of protein applies to rations which allow high protein utilisation; this is appropriate because it is desirable in practice to calculate the protein content of the ration based on the requirements. The lower energy utilisation of ME from fermented carbohydrates compared with carbohydrates digested in the small intestine is based mainly on fermentation heat, according to current knowledge (Susenbeth, 2005). This heat production is included in the factor for DOR because it is both a characteristic which is clearly attributable to the feed, and constant. All energy losses associated with fermentation can therefore be recorded with sufficient accuracy using a digestibility study.

2. The higher energy utilisation of energy from fat is not taken into account at ME level. In the view of the AfBN, this is the only unsolved problem in the energy evaluation of feeds - but not for the purposes of performance prediction.

3. The ME is basically valid for maintenance requirements and all types of performance. In addition, it has the highest compatibility with all other systems since the various NE values are generally derived from the ME or the digestible nutrients.

4. Recent results of digestibility studies conducted in various countries can be adopted independently of the energy evaluation system and used to extend the feed tables. Conversely, published NE values can only be used within the respective system. The methodological details of digestibility studies are harmonized internationally (GfE, 2005).

5. New findings on requirements can be introduced into practice much more easily in the ME system, since they only concern the recommendations for energy supply and do not require corrections to the feed tables.

6.3. Aspects relating to compound feed formulation and feeding practice

Evaluation at ME level is not just one system among others but the common basis of all energy evaluation systems[15]. A ME derived from the content of digestible nutrients for a given feed also allows the calculation of the NE value in the respective systems. The ME therefore ensures compatibility between NE systems.

If experimental verification of a feed value is needed, this can be done on the basis of digestibility studies with additional recording of urinary losses. As a result, the ME value is based on a measurement and is not determined by calculation. Conversely, the NE value of a feed material or feed mixture cannot be tested experimentally with reasonable effort.

Performance prediction has a key role in ration formulation[16] but must not be limited to energy retention. For this purpose, the levels of all relevant ration constituents, the protein quality and the animals' properties must also be known. Combined with this information, energy evaluation of feed at ME level allows a precise and comprehensive performance prediction.

It has been postulated that feed formulation based on NE would lead to lower protein concentrations in compound feed and to a reduction in price since, in contrast to ME, NE classifies low-protein and starch-rich feeds as more beneficial in energy terms and therefore downgrades protein-rich feeds. This is of crucial importance because it has also been postulated that conversion from ME to NE would contribute to a reduction in N emissions. These postulates were extensively tested using practical conditions by a large compound feed manufacturer in cooperation with the AfBN. These tests showed that feed formulation based on ME (GfE, 2008) leads to the same feed material inclusion and thus to identical crude protein concentrations and prices as formulation based on NE (INRA, 2004). In both systems, the minimum price was achieved at the minimum crude protein concentration if amino acid levels were used on the basis of precaecal digestibility. This means that the goal of reducing N emissions is equally achievable in both systems[17].
The NE supply recommendations for different growth stages, sexes and genotypes are not currently available.

15 Because ME differs from DE only by including energy losses via methane and urine, this is also true for DE.

16 In compound feed manufacturing, optimising rations generally means minimising prices while maintaining predetermined concentrations of nutrients. Not before the effects of varying concentrations on all relevant fattening traits (growth, carcass yield, feed intake, nutrient excretion) are considered and thus a prediction of performance is conducted, the term "optimisation" can be justified.

17 For correct comparison of the two systems, it was necessary to carry out the formulations on the basis of the levels of precaecally digestible amino acids and to specify the precisely corresponding NE and ME values as target values of the mixture. Failure to observe these requirements might be the reason why divergent results of such comparisons have been reported on various occasions. However, it is not the task of the energy evaluation system to determine the optimum crude protein concentration of compound feeds. Concentrations of protein and amino acids must be ascertained by target setting derived from the requirements.

In addition to maintenance and growth requirements, the growing pig's needs for physical activity and thermoregulation must be taken into account. For example, there are differences in physical activity between castrated males and boars, and activity is also influenced by housing conditions. However, such requirements can be stated in a physiologically meaningful way only at ME level.

There is therefore no reason, either from a scientific perspective or from the perspective of pig feeding practice, to convert from ME to one of the existing NE systems. According to current knowledge, ration formulation based on ME is therefore to be regarded as best practice.

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Stellungnahme zur energetischen Futterbewertung beim Schwein auf Basis Umsetzbarer Energie versus Nettoenergie

Zusammenfassung

Die vorliegende Stellungnahme ist eine umfassende Darlegung der wissenschaftlichen Argumente sowie der für die praktische Fütterung und Rationsgestaltung relevanten Aspekte, die bei der Futterbewertung beim Schwein auf der Basis der Umsetzbaren Energie (ME) und der Nettoenergie (NE) von Bedeutung sind.

Die ME gibt das Vermögen eines Futtermittels an, dem Stoffwechsel des Tieres Energie bereitzustellen. Die NE berücksichtigt darüber hinaus die im Stoffwechsel auftretenden, nährstoffspezifischen Wärmeverluste und gibt die beim wachsenden Tier retinierte Energie (RE) an. Da die Höhe dieser Verluste insbesondere beim Protein jedoch nicht konstant ist, und der Bedarf für den Grundumsatz sowie für die Trächtigkeit, Thermoregulation und Bewegungsaktivität als Äquivalent zur RE angegeben werden muss, ergeben sich daraus Probleme bzw. Ungenauigkeiten. Die RE als Teil der Leistungsvorhersage gelingt mit einem NE-System daher nur begrenzt. Erst wenn die Zusammensetzung der Ration, insbesondere deren Protein- und Aminosäurengehalte vorliegen sowie die Eigenschaften des Tieres, vor allem dessen Proteinansatzvermögen, bekannt sind, ist eine Vorhersage des Wachstums, der Körperzusammensetzung und der ausgeschiedenen Nährstoffe möglich.

Umfangreiche Berechnungen, die in Zusammenarbeit mit einem großen Futterhersteller durchgeführt wurden, haben ergeben, dass Mischfutteroptimierungen auf der Basis NE gegenüber ME nicht zu einer Kostenreduktion und auch nicht zu einer Absenkung des Proteingehalts führen. Ein einzelnes NE-System hat darüber hinaus den Nachteil, dass es mit anderen NE-Systemen nicht kompatibel ist, während die NE-Werte verschiedener NE-Systeme sich in der Regel aus der ME berechnen lassen. Ein ganz erhebliches Problem wird auch darin gesehen, dass die Möglichkeit einer experimentellen Überprüfung von NE-Werten aufgrund des hohen Aufwands für Respirationsversuche nicht gegeben ist. Die ME hingegen lässt sich mit Hilfe eines Verdaulichkeitsversuchs, ergänzt durch eine Harnsammlung, bestimmen. Zukünftige neue Erkenntnisse über die Effizienz der Nährstoffverwertung und den Bedarf können in einem ME-System bei den Versorgungsempfehlungen einfacher und rascher berücksichtigt werden als in NE-Systemen, da hier die Futterwerte und damit auch Tabellenwerke geändert werden müssen.

Es ist daher weder aus wissenschaftlicher Sicht sinnvoll noch im Interesse der landwirtschaftlichen Praxis, eine Umstellung von der ME auf eines der vorhandenen NE-Systeme vorzunehmen. Eine Rationsgestaltung auf Basis der ME ist nach heutigen Wissensstand als ‚best practice' anzusehen.

1. Einleitung

Die Bedeutung einer präzisen Bestimmung des Energielieferungsvermögens von Futtermitteln ergibt sich daraus, dass der ökonomische Wert der meisten Futtermittel überwiegend vom Energiegehalt abhängt und die Leistung eines Tieres in hohem Maße von der Energieversorgung beeinflusst ist. Bei Futtermitteln mit geringen Fettgehalten ist vor allem ihr Gehalt sowie die Verdaulichkeit der Faserfraktionen für die Variation des energetischen Futterwert bestimmend, da Proteine, Stärke und Zucker in der Regel eine hohe praecaecale Verdaulichkeit (pcV) aufweisen. Die bei der Fermentation der Faser auftretenden Verluste in Form von Methan, Fermentationswärme und mikrobieller Substanz verringern des Weiteren den energetischen Wert faserreicher Futtermittel. Folgt man dem Postulat von Menke (1987), dass der energetischen Futterbewertung die Aufgabe zukommt, die überwiegend durch Eigenschaften des Futters bedingten Verluste zu schätzen, sind daher zusätzlich zu den Verlusten durch die Ausscheidung über den Kot diejenigen über die Methanbildung und den Harn zu berücksichtigen (Umsetzbare Energie, ME). Wird darüber hinaus die durch Nährstoffzufuhr bedingte Wärmebildung im Stoffwechsel einbezogen, führt dies zur Nettoenergie (NE). Gegenstand des vorliegenden Beitrags ist es, den aktuellen Stand der energetischen Futterbewertung beim Schwein in Deutschland darzustellen und die Gründe aufzuzeigen, die zur Entscheidung führten, die Bewertung auf der Stufe der ME vorzunehmen. Des Weiteren werden mögliche Vorteile und Grenzen einer Bewertung auf der Stufe der NE dargelegt sowie damit verbundene Probleme angesprochen. Mit dem vorliegenden Text erweitert der Ausschuss für Bedarfsnormen (AfBN) der Gesellschaft für Ernährungsphysiologie (GfE) die Argumentation, die er bereits in Form einer kurzen Pressemitteilung im Dezember 2015 publiziert hat[1]. Der Text basiert auf den Ausführungen der GfE (2006; Kapitel 2), den Publikationen bzw. Tagungsbeiträgen von Susenbeth (2005, 2010 und 2016), in denen auch Einzelaspekte näher behandelt werden.

1 (http://www.gfe-frankfurt.de/download/GfE_Nettoenergie_Schwein.pdf)

2. Die Bewertung auf der Stufe der Umsetzbaren Energie

Es war der Leitgedanke des AfBN, den energetischen Futterwert als das Potential zu definieren, Energie dem Stoffwechsel des Tieres zur Verfügung zu stellen, und diesen aus den Gehalten an verdaulichen (Roh-) Nährstoffen zu berechnen (GfE, 2006). Dies führt notwendigerweise zu einer Bewertung auf der Stufe der ME. Hingegen kann die Voraussage der Leistungen und damit auch der retinierten Energie (RE) nicht primäre Aufgabe der energetischen Futterbewertung sein, da die Aminosäurenversorgung, das Leistungspotential und die Art der Leistung der Tiere ebenfalls auf die Verwertung der Energie Einfluss nehmen, so dass eine Leistungsvorhersage allein aufgrund der Eigenschaften eines Futtermittels nur sehr begrenzt möglich ist (siehe die Ausführungen im Folgenden).

Die Formel zur Berechnung des Gehalts an ME lautet (GfE, 2006):

ME = 20,5 DXP + 39,8 DXL + 17,3 ST + 16,0 ZU + 14,7 DOR (kJ bzw. g/kg Trockenmasse [TM]),

wobei DXP verdauliches Rohprotein, DXL verdauliches Rohfett, ST Stärke, ZU Zucker, DOR verdaulicher organischer Rest (DOR = DOS - DXP - DXL - ST - ZU; DOS = verdauliche organische Substanz) bedeuten. Diese Formel basiert auf den umfangreichen Untersuchungen der Rostocker Arbeitsgruppe (Jentsch et al., 2001). Lediglich der Faktor für DOR wurde gegenüber der ursprünglichen Formel modifiziert. Mit diesem werden die Methanverluste und die Fermentationswärme berücksichtigt. Diese Berechnungsgleichung ist sehr robust und sicher in ihrer Anwendung, da die Faktoren mit den theoretisch zu erwartenden Werten fast identisch sind. Der Faktor 20,5 für DXP entspricht einem ME-Wert für Protein, wenn dieses effizient im Stoffwechsel genutzt wird, d.h. die N-Ausscheidung über den Harn gering ist. Zu beachten ist, dass die Faktoren für ST und ZU sich nicht auf die verdaulichen Anteile beziehen, sondern auf den Gesamtgehalt im Futter; aufgrund ihrer in der Regel sehr hohen pcV sind diese Faktoren jedoch nahezu mit dem Brennwert identisch.

Die Berücksichtigung des DOR, mit der die Faserfraktion sowie andere Nicht-Stärke-Polysaccharide (NSP) nicht analytisch, sondern rechnerisch ermittelt werden, führt zu einem erheblichen Vorteil hinsichtlich der Sicherheit bei der Anwendung der Gleichung[2]. Analytische Fehler, insbesondere bei den Kohlenhydratfraktionen, wirken sich nur geringfügig auf den berechneten ME-Wert aus, da eine Über- oder Unterschätzung von Nährstoffgehalten quantitativ durch den DOR wieder aufgefangen wird. Die Faktoren für die drei Fraktionen DOR, ST und ZU unterscheiden sich nicht erheblich, so dass die Folge eines Analysenfehlers für den ME-Wert des Futters gering ist. Wird beispielsweise der Stärkegehalt um 5 Prozentpunkte zu niedrig analysiert, nimmt folglich der Gehalt an DOR um 5 Prozentpunkte zu. Trotz dieses erheblichen analytischen Fehlers beträgt der daraus resultierende Fehler für die Bestimmung des ME-Gehalts des Futtermittels jedoch nur (17,3 kJ/g - 14,7 kJ/g) • 50 g = 0,13 MJ/kg TM. Die GfE (2006) weist darauf hin, dass die Anwendung der Formel für Einzelfuttermittel, die nennenswerte Gehalte beispielsweise an Pektinen oder Alkoholen aufweisen, zu Fehleinschätzungen führen kann. In diesen Fällen kann die Genauigkeit der Berechnung verbessert werden, wenn die Menge und der Energiewert solcher Nährstoffe gesondert berücksichtigt werden und gegebenenfalls die davon betroffene Nährstofffraktion entsprechend verringert wird[3]. Entsprechendes gilt auch für Futtermittel, bei denen die Stärke eine geringe pcV aufweist; die praecaecal nicht verdaute Stärke ist dem DOR zuzurechnen.

Es soll noch die Frage beantwortet werden, ob mit dem Faktor 14,7 für DOR, der zunächst recht hoch erscheint und nur um 2,6 niedriger ist als der für Stärke, alle mit einer Fermentation verbundenen Verluste im erforderlichen Umfang berücksichtigt sind. Diese 2,6 kJ/g erfassen nur die Verluste über Fermentationswärme und Methanbildung. Die bei der Fermentation gebildete mikrobielle Substanz stellt eine weitere und bedeutendere Verlustquelle dar und beträgt über 20 % der fermentierten Energie. Dieser Verlust wird jedoch nicht mit dem Faktor für den Energiewert des DOR berücksichtigt, da im Verdaulichkeitsversuch eine Erfassung der mikrobiellen Substanz in der XP-Fraktion sowie bei anderen Fraktionen über die Kotanalyse schon erfolgt ist. Es wäre daher nicht richtig, aus der Formel abzuleiten, dass 1 g fermentierte Faser 14,7 kJ ME liefert; vielmehr liegt der Energiewert auch nach dieser Formel unter 11,2 kJ ME/g, wenn alle durch Fermentation bedingten Verluste – einschließlich der mikrobiellen Substanz – ausschließlich beim Faktor für DOR berücksichtigt würden.

Mit der Berechnungsgleichung der GfE (2006) für die ME gelingt es sehr gut, die aus dem Futter dem Stoffwechsel zur Verfügung gestellte Energie anzugeben. Eine gewisse Einschränkung ergibt sich aus der Variabilität der Harnenergie. Die Höhe der Harnenergieverluste kann aber nicht dem Einzelfuttermittel zugeordnet werden, weil diese Verluste überwiegend ein Resultat des Gehalts und der Qualität des Proteins

2 Eine organische Restfraktion wird auch bei Berechnungsgleichungen anderer Autoren berücksichtigt (Susenbeth, 2005). Es ist allerdings zu beachten, dass diese Fraktion in den jeweiligen Gleichungen unterschiedlich definiert bzw. berechnet wird.

3 In entsprechender Weise wird bei der Formel F.V09 des CVB (2016) vorgegangen.

der Gesamtration sind, welche durch die pcV und das Muster der Aminosäuren bestimmt wird, sowie vom Proteinansatz der Tiere abhängen.[4]

3. Die Leistungsvorhersage auf der Basis der Umsetzbaren Energie

Die energetische Futterbewertung kann nicht losgelöst von den Fragen der Bedarfsermittlung bzw. der Leistungsvorhersage vorgenommen werden (Menke, 1987). Es ist zwar nicht die eigentliche Aufgabe der energetischen Futterbewertung, eine Leistungsvorhersage vorzunehmen. Der energetische Futterwert muss jedoch so definiert werden, dass er als einer der Determinanten für die Leistungsvorhersage verwendet werden kann. So können für die Voraussage der Energieretention (RE) beim Wachstum die von Jentsch et al. (2000a) angegebenen und durch umfassende Experimente gut abgesicherten Verwertungsfaktoren (k = RE/ME) verwendet werden. Sie betragen für Stärke, Protein und Fett 0,757, 0,623 bzw. 0,859 und, wenn Stärke = 100 gesetzt wird, für Protein und Fett 82 bzw. 113 %[5]. Vereinfachend kann jedoch *in praxi* für die Voraussage der RE als Stoffansatz im Körper und als Milch aus der ME der Ration ein mittlerer k_{pf}-Wert von 0,72 - 0,74 bzw. kl-Wert von 0,72 (Susenbeth, 19966; GfE, 2006) verwendet werden, wobei dann die Unterschiede in der Effizienz der Verwertung zwischen den Nährstoffen nicht berücksichtigt werden.7,8 Dies bedeutet, dass die Voraussage der RE allein aus der ME ohne Kenntnis der Gehalte der jeweiligen Nährstoffe mit einer gewissen Ungenauigkeit verbunden sein muss. Dies ist der entscheidende Tatbestand, der als Argument für die Notwendigkeit der Futterbewertung auf der Stufe der NE herangezogen wird. Die Ungenauigkeit, die man bei einer pauschalierenden Berechnung der RE aus der ME in Kauf nimmt (konstanter k-Wert, keine Berücksichtigung der Nährstoffunterschiede), ist für eine Situation bei bedarfsgerechter Proteinversorgung jedoch sehr gering. Ähnlich verhält es sich beim Fett: die Energieverwertung nimmt pro Prozentpunkt Fett in der Ration nur um 0,001 zu.

Bei Annahme einer konstanten9, von der Nährstoffzusammensetzung unabhängigen Verwertung der ME nimmt die Genauigkeit der RE-Berechnung eigentlich nur bei Rationen mit höheren Fettgehalten und bedarfsüberschreitenden Proteingehalten etwas ab, und die NE dürfte in diesem Fall einen Vorteil aufweisen. Für eine präzisere Schätzung der RE, die zudem die Kenntnis des Proteinansatzes des Tieres sowie des Gehalts und der Qualität des Proteins der Ration zur Voraussetzung hat und gegebenenfalls auch Einflüsse der Haltung berücksichtigen muss, stellt die Bewertung der Energielieferung der Futtermittel nach ME keinerlei Hindernis dar, vielmehr kann und sollte sie auf dieser Grundlage erfolgen. Damit ist das Argument, dass eine genauere Schätzung der Energieretention mit der NE erfolge, für die praktische Rationsgestaltung und Fütterung ohne Belang.

4. Die energetische Bewertung auf der Stufe der Nettoenergie

Das zentrale Anliegen, die energetische Futterbewertung auf der Stufe der NE vorzunehmen, ist, wie oben erwähnt, die Voraussage der RE aufgrund der Berücksichtigung der unterschiedlichen *energetischen Verwertung der einzelnen Nährstoffe*. Eine kritische Würdigung der NE ist daher zuerst vor dem Hintergrund dieser Zielstellung vorzunehmen:

1. Die NE gibt die RE (überwiegend als Fettansatz) an, die aus dem ME-Gehalt der einzelnen Nährstoffe und den jeweiligen Verwertungsfaktoren berechnet wird. Der so abgeleitete RE-Wert ist jedoch mit einem grundsätzlichen Problem verbunden, das vor allem beim Protein offensichtlich wird. Es muss davon ausgegangen werden, dass die **Effizienz der Verwertung der ME aus Protein** nicht konstant und für den Proteinansatz eine andere ist als bei einer rein energetischen Nutzung. Daher kann der energetische Wert des

4 Von diesem Problem der Variabilität der Ausscheidung von Energie über den Harn ist die NE in gleicher Weise betroffen. Die Möglichkeit, die Genauigkeit des ME-Werts (und damit auch des NE-Werts) einer Ration zu verbessern und diese Unschärfe zu vermeiden, besteht in der Berücksichtigung der Proteinverwertung des Tieres, welche die Hauptursache für Unterschiede in der Ausscheidung von Energie über den Harn ist. Ein Vorschlag für eine derartige Berechnung wurde erarbeitet (A. Susenbeth, unveröffentlicht), bei der zwei Energiewerte für das Protein verwendet werden: Einen höheren Wert für Protein, das für den Proteinansatz verwendet wird und die maximale Proteinverwertung des Idealen Proteins aufweist, und einen deutlich niedrigeren für Protein, das über den Minimalbedarf (d.h. die Mindestversorgung an Rohprotein; s. Tab. 4.9; GfE, 2006) hinausgeht und dessen Stickstoff vollständig über den Harn ausgeschieden wird.

5 Die Verwendung der von Noblet (2006) angegebenen Verwertungskoeffizienten führt zu abweichenden Ergebnissen, da sich diese nicht unerheblich von denjenigen bei Jentsch et al. (2000a) unterscheiden.

6 Der hier angegebene höhere k_{pf}-Wert gegenüber der Originalarbeit ergibt sich aus der Verwendung der Energiewerte von 23,8 kJ/g Rohprotein und 38,7 kJ/g Rohfett.

7 Von der unterschiedlichen Energieverwertung der einzelnen Nährstoffe sind der k_p-Wert (= 0,56) bzw. k_f-Wert (= 0,74) (GfE, 2006) zu unterscheiden. Diese geben die Effizienz der ME-Verwertung für den Energieansatz in Form von Protein bzw. Fett an, und zwar unabhängig von der Nährstoffzusammensetzung der Ration, d.h. sie haben nicht die Herkunft der Energie, sondern deren Verwendung im Auge und gelten daher für Rationen, die übliche Gehalte an Protein, Faser sowie Fett aufweisen.

8 Ein k_{pf}-Wert von 0,73 - 0,74 ergibt sich auch nach INRA (2004; Gl. NE7) für eine mittlere Rationszusammensetzung.

9 Siehe Fußnote7.

Proteins durch Faktoren beeinflusst sein, die nicht von Eigenschaften des Futtermittels verursacht sind und bei der Bewertung nicht bekannt sein können. Erst die Kenntnis der Gesamtration, der Qualität des Proteins sowie des Leistungsvermögens des Tieres ermöglicht die Voraussage des Proteinansatzes und damit auch der Effizienz der Proteinverwertung. So weist erneut das niederländische Forschungsinstitut Schothorst Feed Research (2016) darauf hin, dass die Hauptbeschränkung von NE-Systemen darin bestehe, dass die post-absorptive Nährstoffnutzung nicht berücksichtigt werden kann und daher die Verwendung eines einheitlichen Energiewerts für das Protein die unterschiedlichen Verwendungsmöglichkeiten unberücksichtigt lasse. Boisen (2007), der das dänische System, die potentielle physiologische Energie, entwickelt hat, resümiert sogar, dass die NE keine geeignete Basis für eine Futterbewertung darstelle. Er greift die schon früher von anderen Autoren vorgebrachte Kritik auf, dass eine NE nur für eine spezifische Leistung gelte, und dass der Response des Tieres in Form von RE als Maß für den Futterwert auch deshalb ungeeignet sei, weil gerade die vom Tier selbst ausgehenden Einflüsse zu einer Variabilität eines solchen Futterwerts führen.
Die durch diese Variabilität der Proteinverwertung bedingte nicht mögliche Festlegung eines generell zutreffenden Energiewerts für Protein spricht für eine Bewertung auf der Stufe ME, da dieses Problem bei einer Futterbewertung nach ME eben nicht berücksichtigt werden muss – oder offengelassen wird. In zwei bedeutenden NE-Systemen, dem Rostocker Futterbewertungssystem (Beyer et al. 2003, basierend auf Jentsch et al., 2001) und dem Französischen System (INRA, 2004, basierend auf Noblet et al., 1994) ist offensichtlich der Energiewert von Protein für eine rein energetische Nutzung angegeben. Sollten in Zukunft abgesicherte spezifische Verwertungsfaktoren zur Verfügung stehen, die für die jeweilige Proteinverwendung gelten (Proteinansatz bzw. energetische Nutzung), kann dies bei der NE nur mit großem Aufwand berücksichtigt werden, da die Futterbewertung geändert und Futtermitteltabellenwerke umgearbeitet werden müssten. Bei der ME jedoch ist eine Berücksichtigung relativ einfach im Rahmen der Bedarfsermittlung oder Leistungsvorhersage möglich. – Es sind neben der Variabilität der Verwertung weitere konzeptionelle Probleme bei der Bewertung auf der Stufe der NE vorhanden:

2. Die etablierten NE-Werte sind zunächst nur für die Leistung als Stoffansatz (RE) im Wachstum gültig. Die **Milchbildung** weist zwar eine ähnliche Effizienz der ME-Verwertung auf. In einem NE-System müsste jedoch für die Lactosebildung ein gesonderter Verwertungsfaktor eingeführt werden. Noch offensichtlicher wird das Problem, wenn der Bedarf für die **Trächtigkeit** bestimmt werden soll: Da die Verwertung der ME für Trächtigkeit deutlich geringer ist als für das Wachstum, übersteigt die Bedarfsangabe für Trächtigkeit – angegeben als NE für Wachstum – die tatsächliche Energieretention in Föten und Adnexen um ein Mehrfaches. Eine formale Umrechnung ist zwar möglich, aber nur schwer zu vermitteln.

3. Ein noch größeres Problem tritt beim Bedarf für **Erhaltung** auf, da dieser ebenfalls als RE angegeben werden muss. Immerhin entfällt auch beim intensiv wachsenden Tier ungefähr ein Drittel des Gesamtbedarfs an Energie auf den Erhaltungsumsatz. Es ist bekannt, dass sich nicht nur die Effizienz der Verwertung im Erhaltungsumsatz von der beim Wachstum unterscheidet, sondern dass auch die Relationen der Verwertungsgrößen zwischen Nährstoffen andere sind als beim Wachstum (Chudy und Schiemann, 1969; Blaxter, 1989; Jentsch et al., 2000a).

4. Der Bedarf für **körperliche Aktivität** und **Thermoregulation** *kann nur* in ME angegeben werden, da die hierfür aufgewendete ME vollständig in Wärme umgewandelt wird und NE-Werte für einen solchen Bedarf nicht existieren. Die Wärmeproduktion ist größer als der hierfür erforderliche Bedarf an NE. Ein solcher Bedarf, der nicht auf Erhaltung und Wachstum entfällt, kann unter praktischen Haltungsbedingungen beim Mastschwein 15 % des Gesamtbedarfs betragen (Naatjes et al., 2014). Es wurde außerdem beobachtet, dass eine faserreiche Fütterung die körperliche Aktivität reduziert. Dadurch kommt es zu einer geringeren durch Aktivität verursachten Wärmeproduktion, welche die erhöhte Fermentationswärme kompensiert (Rijnen, 2003), wodurch der gemessene RE-Wert nicht dem NE-Wert der Faser entspricht. Solche Effekte sind bei der NE-Berechnung zu berücksichtigen, da dies sonst zu einer Vermischung von Bedarfsbestimmung und Futterbewertung führt.

5. Es besteht ein **methodisches Problem bei der experimentellen Bestimmung des NE-Werts**. Um die RE eines Futtermittels zu bestimmen, müssen Respirationsmessungen als Zulageversuche durchgeführt werden. Da aufgrund des großen Aufwands darauf in der Regel verzichtet wird, ist eine Annahme für die

Höhe des Grundumsatzes10 erforderlich; dieser Wert wird zum gemessenen RE-Wert addiert. Da dieser angenommene Wert für die verschiedenen NE-Systeme nicht identisch ist und der tatsächliche Grundumsatz auch durch Versuchsbedingungen, insbesondere von der Dauer des Nahrungsentzugs beeinflusst sein kann, stellt dieses Problem einen entscheidenden, grundsätzlichen Kritikpunkt an NE-Systemen dar. Dies hat auch zur Folge, dass sich NE-Werte zwischen verschiedenen NE-Systemen unterscheiden, wodurch eine Umrechnung zwischen NE-Systemen erhebliche Schwierigkeiten bereitet. Sollten z. B. neuere Informationen zum Erhaltungsbedarf bzw. Grundumsatz zur Verfügung stehen, könnten diese nicht bei den Versorgungsempfehlungen berücksichtigt werden, sondern müssten zu neuen NE-Werten der Futtermittel und auch aus diesem Grunde zu einer Überarbeitung der Futterwerttabellen führen.

6. Eine **experimentelle Überprüfbarkeit von NE-Werten** ist theoretisch zwar möglich, jedoch nicht realistisch. Zum einen, weil der im jeweiligen System festgelegte Wert für den Erhaltungs- bzw. Grundumsatz übernommen werden muss, also keinen Messwert darstellt, zum anderen, weil zur Zeit nur wenige Institutionen über Respirationskammern für Großtiere verfügen. Man kann den für die ME-Ermittlung ausreichenden Verdaulichkeitsversuch, der durch eine Harnsammlung ergänzt werden kann, zum Zwecke der Futterbewertung noch als vertretbar ansehen, während für die NE-Ermittlung notwendige Gesamtstoffwechselversuche kaum in Betracht kommen.

5. Aktuelle Nettoenergie-Systeme

Eine Beurteilung der energetischen Futterbewertung nach NE muss neben den zuvor erläuterten generellen Aspekten auch die Unterschiede zwischen den jeweiligen NE-Systemen ins Auge fassen. Es seien hier drei aktuelle NE-Systeme11 aufgeführt, welche die RE im Wachstum zur Grundlage haben: INRA (2004), CVB (2016) und das Rostocker System (Beyer et al., 2003). Die jeweiligen Formeln lauten:

Rostocker System (Beyer et al., 2003)

NE = 11,0 DXP + 34,0 DXL + 12,7 ST + 11,6 ZU + 12,0 DOR

INRA (2004)

NE = 12,1 DXP + 35,0 DXL + 14,3 ST + 11,9 ZU + 8,6 DOR

CVB (2016)[12]

NE = 11,70 DXP + 35,74 DXL + 14,14 ST + 12,73 pcv ZU + 9,74 · fermentierte Kohlenhydrate[13]

Es seien hier einige zentrale Aspekte dieser Gleichungen angesprochen. Der Faktor für DXP unterscheidet sich zwar zwischen den NE-Systemen, zeigt aber doch eine bemerkenswert gute Übereinstimmung. Auch für DXL sowie für ZU kann diese Feststellung getroffen werden. Der angegebene Wert für DOR im Rostocker System gilt für Rationen, die eine Energieverdaulichkeit über 80 % aufweisen; liegt jedoch die Energieverdaulichkeit beispielsweise nur bei 60 oder 65 %, beträgt der Faktor 9,2 bzw. 9,9 und ist damit in der Nähe der anderen Systeme. Dies zeigt aber auch, dass der Energiebeitrag dieser Fraktion nach INRA (2004) und CVB (2016) zumindest für übliche energiereiche Rationen eher unterbewertet wird. Wegen der hohen Gehalte an ST in vielen Futtermitteln kommt den Unterschieden im Faktor für ST jedoch die größte Bedeutung zu. Aufgrund von Respirationsversuchen, in denen der Effekt einer Zulage von reiner Stärke auf den Energieansatz gemessen wurde, sind NE-Werte über 14 kJ pro g Stärke als unrealistisch hoch anzusehen, vielmehr liegt die energetische Verwertung von Stärke übereinstimmend im Bereich von 75 - 76 %[14]. Die häufig gemachte Aussage, dass Protein im NE-System nach INRA unterbewertet wird, ist daher so nicht richtig. Vielmehr liegt eine erhebliche Überbewertung der Stärke vor, die mit einer relativen Abwertung der anderen Nährstoffe verbunden ist. Da für die Auswahl der Einzelkomponenten bei der Mischfutteroptimierung nicht die absoluten Energiewerte der Nährstoffe, sondern nur die relativen relevant sind, werden stärkereiche vor allem gegenüber protein- und faserreichen Komponenten überbewertet.

10 Der Grundumsatz ist definiert als die Wärmeproduktion ohne Nahrungsaufnahme und entspricht daher der im Hunger mobilisierten Körpersubstanz (negative RE). Aus diesem Grunde gibt es auch keinen Erhaltungsbedarf an NE, da der Erhaltungsumsatz der Zufuhr an ME entspricht, die erforderlich ist, die Mobilisierung von Körpersubstanz zu verhindern. Der Erhaltungsumsatz ist daher größer als der Grundumsatz.

11 Der „potentielle physiologische Energiewert" des dänischen Systems (Tybirk et al., 2006; Boisen, 2007) ist aus dem ATP-Bildungsvermögen der Nährstoffe abgeleitet. Die Intention dieses Systems stimmt mit der der ME als Energielieferungsvermögen des Futters überein. Die jeweiligen Energiewerte der Nährstoffe zeigen eine Nähe zu NE-Werten, sind aber mit diesen nicht identisch, weshalb sie hier nicht zum Vergleich aufgeführt sind.

12 Formel V.F10; (Formel V.F09 berücksichtigt zusätzlich flüchtige Stoffe und Glycerin.)

13 Summe an verdaulichen NSP, fermentierten ZU und fermentierter ST

14 Nahezu identische Werte wurden bei der Ratte mit 75,5 (Nehring et al., 1961) und am Menschen mit 75,8 (Jentsch et al., 2000b) ermittelt.

6. Zusammenfassende Bewertung

6.1. NE-Systeme

Die Vorzüge und Grenzen der Bewertung von Futtermitteln auf Basis ihres NE-Gehalts lassen sich wie folgt zusammenfassen:

1. Mit den vorliegenden NE-Systemen wird die Energieretention bei Rationen mit hohen (d.h. über die Mindestversorgung hinausgehenden) Proteingehalten und bei fettreichen Futtermitteln genauer vorhergesagt.

2. Der Energiewert von Stärke wird durch die NE nach INRA (2004) und CVB (2016) überbewertet.

3. Der Bedarf für Erhaltung, Trächtigkeit, Milchbildung, Bewegungsaktivität und Thermoregulation muss in NE-Wachstum umgerechnet werden, wodurch die Verständlichkeit leidet und die Vermittelbarkeit erheblich erschwert wird.

4. Die verschiedenen NE-Systeme liefern unterschiedliche Werte für dasselbe Futtermittel und sind nicht kompatibel.

5. Die Möglichkeit zur experimentellen Überprüfung eines Futterwerts ist wegen des extrem hohen Aufwands als nicht realistisch anzusehen.

6. Bei einer Berücksichtigung neuer Erkenntnisse zum Energieumsatz in NE-Systemen müssen die Futterwerttabellen geändert werden. Dies betrifft insbesondere den Energiewert von Protein, der als variabel anzusehen ist, sowie den Grund- bzw. Erhaltungsumsatz.

6.2. ME-System

Die Vorzüge und Grenzen der Bewertung von Futtermitteln auf Basis ihres ME-Gehalts lassen sich wie folgt zusammenfassen:

1. Die ME beschreibt die dem Stoffwechsel zur Verfügung gestellte Energie. Eine gewisse Einschränkung ist dadurch gegeben, dass die Energieverluste über den Harn auch von der Proteinqualität und dem Proteinansatz des Tieres abhängen. Nur ein System auf der Stufe der Verdaulichen Energie (DE) wäre davon nicht betroffen, aber ebenfalls jedes NE-System. Die ME verzichtet damit auf die Berücksichtigung von Unterschieden in der energetischen Verwertung der Nährstoffe. Der ME-Wert des Proteins gilt für Rationen, die eine hohe Proteinverwertung ermöglichen; dies ist durchaus zweckmäßig, da für die Praxis eine Bemessung des Proteingehalts der Ration am Bedarf erwünscht ist. Die geringere energetische Verwertung der ME aus fermentierten gegenüber im Dünndarm verdauten Kohlenhydraten beruht nach heutigem Kenntnisstand überwiegend auf der Fermentationswärme (Susenbeth, 2005). Diese Wärmebildung ist beim Faktor für DOR berücksichtigt, weil sie sowohl eine eindeutig dem Futter zuzurechnende Eigenschaft als auch konstant ist. Mit einem Verdaulichkeitsversuch sind damit alle mit der Fermentation verbundenen Energieverluste ausreichend genau erfassbar.

2. Die höhere energetische Verwertung von Energie aus Fett wird auf der Stufe der ME nicht berücksichtigt, und stellt damit aus Sicht des AfBN das einzig ungelöste Problem bei der energetischen Bewertung von Einzelfuttermitteln dar, – nicht jedoch für die Leistungsvorhersage.

3. Die ME ist grundsätzlich für den Erhaltungsumsatz und alle Leistungsrichtungen gültig. Darüber hinaus weist sie die höchste Kompatibilität mit allen anderen Systemen auf, da die verschiedenen NE-Werte in der Regel aus der ME bzw. den verdaulichen Nährstoffen abgeleitet werden.

4. Neuere Ergebnisse aus Verdaulichkeitsversuchen, die in verschiedenen Ländern durchgeführt wurden, können unabhängig vom Energiebewertungssystem übernommen und zur Erweiterung der Futterwerttabellen genutzt werden, wohingegen publizierte NE-Werte immer nur innerhalb des jeweiligen Systems zu verwenden sind. Die Durchführung von Verdaulichkeitsversuchen ist international abgestimmt (GfE, 2005).

5. Neue Erkenntnisse zum Bedarf können im ME-System wesentlich leichter in die Praxis eingeführt werden, da diese nur die Empfehlungen zur Versorgung betreffen und Korrekturen der tabellierten Futterwerte nicht erforderlich machen.

6.3. Aspekte hinsichtlich Mischfutteroptimierung und Fütterungspraxis

Die Bewertung auf der Stufe der ME ist nicht ein System neben anderen, sondern die gemeinsame Basis aller Energiebewertungssysteme[15]. Eine für ein bestimmtes Futtermittel aus dem Gehalt an verdaulichen Nährstoffen abgeleitete ME erlaubt auch die Berechnung des NE-Werts in den jeweiligen Systemen. Dadurch ist eine Kompatibilität zwischen NE-Systemen über die ME gegeben.

Falls die experimentelle Überprüfung eines Futterwerts erforderlich ist, kann dies auf der Basis eines Verdaulichkeitsversuchs mit zusätzlicher Erfassung der Harnverluste erfolgen, wodurch der ME-Wert auf einer Messung basiert und nicht durch Berechnung bestimmt wird. Der NE-Wert eines Futtermittels oder einer Futtermischung lässt sich hingegen nicht mit vertretbarem Aufwand experimentell überprüfen.

Die Leistungsvorhersage nimmt eine Schlüsselstellung bei der Rationsgestaltung[16] ein, sie darf jedoch nicht auf die Energieretention beschränkt werden. Dazu müssen zusätzlich die Gehalte aller relevanten Inhaltsstoffe der Ration, die Proteinqualität sowie Eigenschaften der Tiere bekannt sein. Zusammen mit diesen Informationen ermöglicht die energetische Bewertung des Futters auf der Stufe der ME eine präzise und umfassende Leistungsvorhersage.

Es wurde postuliert, dass eine Futteroptimierung auf der Basis NE zu geringeren Proteingehalten im Mischfutter und zu einer Reduktion des Preises führe, da im Gegensatz zur ME proteinarme und stärkereiche Futtermittel durch die NE energetisch günstiger und damit proteinreiche ungünstiger eingestuft werden. Dies sei deshalb von besonderer Bedeutung, weil damit eine Umstellung auf NE auch zu einer Reduktion der N-Emissionen beitrage. Diese Postulate wurden unter den praktischen Bedingungen eines großen Mischfutterherstellers in Zusammenarbeit mit dem AfBN umfassend geprüft. Hieraus ergab sich, dass eine Optimierung auf der Basis ME (GfE, 2006) zu gleichen Futterkomponentenanteilen und damit zu identischen Rohproteingehalten und Preisen führt wie eine Optimierung nach NE (INRA, 2004). In beiden Systemen wurde das Optimum bzw. Preisminimum beim Mindestrohproteingehalt erzielt, wenn die Aminosäurengehalte auf der Basis praecaecaler Verdaulichkeit verwendet wurden. Das heißt, in beiden Systemen ist das Ziel der Reduktion der N-Ausscheidungen gleichermaßen zu erreichen[17].

Empfehlungen zur Versorgung mit NE für unterschiedliche Wachstumsabschnitte, Geschlechter und Genotypen stehen zur Zeit nicht zur Verfügung. Beim wachsenden Schwein ist neben dem Bedarf für Erhaltung und Wachstum auch der Energiebedarf für Aktivität und Thermoregulation zu berücksichtigen. So sind Unterschiede in der körperlichen Aktivität zwischen Kastraten und Ebern vorhanden, auch die Haltung hat einen Einfluss auf die Aktivität. Ein solcher Bedarf kann jedoch physiologisch sinnvoll nur auf der Stufe der ME angegeben werden.

Es gibt daher weder aus wissenschaftlicher Sicht noch aus Sicht der landwirtschaftlichen Praxis einen Grund für eine Umstellung von der ME auf eines der vorhandenen NE-Systeme. Eine Rationsgestaltung auf Basis ME ist daher nach dem heutigen Wissensstand als ‚best practice' anzusehen.

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15 Da sich die ME gegenüber der DE nur durch die zusätzliche Berücksichtigung der Energieverluste über Methan und Harn unterscheidet, trifft dies auch für die DE zu.

16 In der Praxis der Mischfutterherstellung wird unter Rationsoptimierung i.d.R. die Preisminimierung bei vorgegebenen, zu erreichenden Nährstoffgehalten verstanden. Erst die Berücksichtigung von Effekten abweichender Gehalte auf verschiedene relevante Merkmale der Mast (Wachstum, Schlachtkörperwert, Futteraufwand, Nährstoffausscheidungen) und damit eine Vorhersage der Leistungen rechtfertigen eigentlich den Begriff der ‚Optimierung'.

17 Für einen korrekten Vergleich der beiden Systeme war es erforderlich, die Optimierungen auf der Basis der Gehalte an pcv Aminosäuren vorzunehmen und die sich exakt entsprechenden NE- und ME-Werte als Zielgrößen der Mischung vorzugeben. Die Nichtbeachtung dieser Voraussetzungen könnte der Grund dafür sein, dass verschiedentlich über abweichende Ergebnisse solcher Vergleiche berichtet wurde. Jedoch ist es eigentlich nicht Aufgabe des Energiebewertungssystems, den Rohproteingehalt des Mischfutters zu bestimmen. Der Gehalt an Protein und Aminosäuren ist durch eine aus dem Bedarf abgeleitete Vorgabe bei der Optimierung festzulegen.

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Stellungnahme zur Unerlässlichkeit von Tierversuchen und zur Eignung von Ersatzmethoden in der Tierernährungsforschung

Synopse

Unsere Gesellschaft hat hohe Erwartungen an die globale Ernährungssicherheit, die Futtermittel- und Lebensmittelsicherheit sowie den damit verbundenen Schutz der Nutztiere, der natürlichen Ressourcen und der Umwelt. Diesen Erwartungen gerecht zu werden setzt Forschungsaktivitäten der Tierernährung voraus. In der Tierernährungsforschung werden sowohl Tierversuche durchgeführt als auch Ersatzmethoden entwickelt und verwendet. Stehen geeignete Ersatzmethoden nicht zur Verfügung, sind Tierversuche unerlässlich. Planung und Durchführung von Tierversuchen folgen der Maxime, Belastungen der Versuchstiere auf ein möglichst geringes Maß zu beschränken.

Die Haltung und die Nutzung von Tieren durch den Menschen sind Bestandteil des gesellschaftlichen Handelns und Gegenstand öffentlicher Diskussionen. Die Vorstellungen zur Nutztierhaltung entfalten daher Konsequenzen für die wissenschaftliche Arbeit an und mit Tieren und für die Rahmenbedingungen, unter denen sich Forschungstätigkeit entwickelt.

Tierversuche werden von Teilen der Gesellschaft kritisch gesehen oder gänzlich abgelehnt. Allerdings sind Tierversuche trotz aller Erfolge in der Entwicklung und Etablierung von Ersatzmethoden auch weiterhin unerlässlich. Die Erklärung und Begründung dieser Unerlässlichkeit ist eine Voraussetzung dafür, dass Tierversuche von der Gesellschaft akzeptiert werden. Dabei gehen die Gründe über die intrinsische Motivation und individuelle Verantwortung der Forschenden sowie den gesetzlichen Auftrag zu wissenschaftlicher Forschung hinaus. Maßgeblich ergibt sich die Unerlässlichkeit von Tierversuchen aus vielfältigen Ansprüchen und Erwartungen, die das Ergebnis gesellschaftlicher Diskussionen und daraus folgender politischer Entscheidungen sind.

Das Ziel der vorliegenden Stellungnahme ist es, Erklärungen für die Notwendigkeit von Tierversuchen in der Tierernährungsforschung zu geben. Hierzu werden zunächst die Aufgaben der Tierernährung erläutert. Anschließend wird dargelegt, welche Methoden in der Tierernährungsforschung genutzt werden, und wie diese Methoden auch die Entwicklung und Verbesserung von Ersatzmethoden ermöglichen. Erläuterungen und Reflektionen zu Tierversuchen auch außerhalb der Tierernährungsforschung gibt es z. B. von der Senatskommission für tierexperimentelle Forschung der Deutschen Forschungsgemeinschaft[1] und der Max-Planck-Gesellschaft[2].

1 Senatskommission für tierexperimentelle Forschung der Deutschen Forschungsgemeinschaft (Hrsg.): Tierversuche in der Forschung. www.dfg.de/download/pdf/dfg_im_profil/geschaeftsstelle/publikationen/dfg_tierversuche_0300304.pdf

2 White Paper der Max-Planck-Gesellschaft: Stellungnahme der Max-Planck-Gesellschaft zu Tierversuchen in der Grundlagenforschung. https://www.mpg.de/tierversuche-whitepaper

1. Rahmenbedingungen und Aufgaben der Tierernährungsforschung

Nutztiere sind integraler Bestandteil von landwirtschaftlichen Systemen, Nährstoffkreisläufen und Umwelten. Alle Szenarien für die weltweite Bevölkerungsentwicklung gehen auch für die Zukunft von einer hohen Nachfrage nach Lebensmitteln tierischen Ursprungs aus (Milch, Fleisch, Eier und Fische sowie deren jeweilige Verarbeitungsprodukte). Soll diese Nachfrage gedeckt werden, so wird ein Bündel von Maßnahmen erforderlich, das die Verminderung der Verluste entlang der Produktions- und Verarbeitungskette beinhaltet, aber auch die wissensbasierte Weiterentwicklung der Nutztierhaltung zum Ziel hat. Die Gewinnung von pflanzlichen und tierischen Lebensmittel ist allein schon durch die Größe der landwirtschaftlich nutzbaren Fläche auf der Erde limitiert, von der der überwiegende Teil Dauergrünland ist. Zudem setzt die Erzeugung landwirtschaftlicher Primärprodukte das Vorhandensein von Wasser und Nährstoffressourcen voraus, die global knapp und ungleich verteilt sind, und von denen einige endlich sind. In diesem Spannungsfeld ist die **Sicherung der Ernährung** einer weiter wachsenden Weltbevölkerung eine der großen Herausforderungen für die Zukunft.

Lebensmittel sollen nicht nur in ausreichender Menge und zu akzeptablen Preisen zur Verfügung stehen, sondern außerdem eine hohe Qualität und Sicherheit für den Verbraucher aufweisen. Die Gewährleistung der Lebensmittelsicherheit beginnt mit der Futtermittelsicherheit, weil viele Inhaltsstoffe in Lebensmitteln von Tieren durch die Fütterung beeinflussbar sind. Dies gilt für wertgebende Bestandteile ebenso wie für unerwünschte Stoffe oder auch einige Krankheitserreger, die für den Menschen ein Gefährdungspotenzial haben. Kenntnisse über einen möglichen Transfer von Stoffen und Erregern aus dem Futter über das Tier in das Lebensmittel sind für die **Beurteilung der Lebensmittelsicherheit** somit unverzichtbar. Rechtliche Rahmenbedingungen schreiben daher Tierversuche vor, wenn es z. B. um die Zulassung von Futtermittelzusatzstoffen geht.

Der Stoffwechsel von Mensch und Tier hat biologisch bedingte Grenzen, die unvermeidbar Verluste nach sich ziehen. Viele Nahrungsbestandteile können nicht vollständig verwertet werden, was zur Ausscheidung mit dem Kot und Harn der Tiere, zur Abgabe von Gasen und zur Bildung von Wärme führt. Bei hohen Tierzahlen – in Verbindung mit knapper landwirtschaftlicher Nutzfläche – kann der Anfall von Wirtschaftsdüngern (Gülle, Festmist, Jauche) lokal und regional zu einer Akkumulation von Nährstoffen auf der Fläche und zu unerwünschten Einträgen von beispielsweise Nitrat und Phosphat in Böden und Gewässer führen. Mit der Tierhaltung verbundene Emissionen von Gasen wie Ammoniak und Methan wirken ebenfalls auf die Umwelt. Es gehört mit Recht zu den Erwartungen der Gesellschaft, dass **negative Wirkungen der Tierhaltung auf die Umwelt** soweit wie möglich minimiert werden.

Etablierte Formen der Tierhaltung werden von Teilen der Gesellschaft in Deutschland hinsichtlich der Angemessenheit von Produktionsbedingungen kritisch hinterfragt, teils sogar abgelehnt. **Tiergesundheit und Wohlbefinden der Tiere** stellen ein hohes Gut dar. Maßnahmen zur Gesunderhaltung und zur Ermöglichung eines arttypischen Verhaltens von Tieren, wie z. B. ein hohes Angebot an Grobfutter zum Anregen des Wiederkäuens der Rinder, können jedoch negative Auswirkungen auf die Umwelt und die Produktivität der Tierhaltung haben. Die hier bestehenden **Zielkonflikte** kann die Gesellschaft im Diskurs nur dann lösen oder zumindest entschärfen, wenn die Forschung entsprechende Zusammenhänge aufklärt und die wissenschaftlich begründeten Argumente für ein sinnvolles Handeln liefert.

Im Kontext von globaler Ernährungssicherung, Futtermittel- und Lebensmittelsicherheit sowie Tier- und Umweltschutz hat die **Tierernährung eine zentrale Bedeutung**. Zuvorderst stellt sie die bedarfsgerechte Versorgung der Tiere als Grundvoraussetzung für Gesundheit, Wohlbefinden und Leistung sicher. Mit gezielten Fütterungsmaßnahmen wird zudem angestrebt, die Nährstoffverwertung zu verbessern und damit die Ausscheidungen der Tiere und Einträge in die Umwelt zu vermindern. Futtermittel und Fütterung beeinflussen auch die Gehalte an Nähr- und Geschmacksstoffen in Produkten wie Fleisch, Milch und Eiern, und wirken infolge dessen auch auf die Verarbeitungseigenschaften dieser Produkte ein, weil es einen – stoffspezifisch unterschiedlich stark ausgeprägten – Übergang dieser Stoffe in das Tier und seine Gewebe gibt.

Um Tiere bedarfsgerecht versorgen zu können, muss die Futtermittelbasis mit den Ansprüchen der Tiere in Einklang gebracht werden. Beide Segmente, Futtermittelbasis und Ansprüche der Tiere, **verändern sich jedoch ständig**: Zum einen gibt es ein unterschiedliches, genetisch bedingtes Leistungspotenzial der Tiere, das sich zwischen Tierarten, Rassen, Linien und Nutzungsrichtungen unterscheidet und einen unterschiedlichen Bedarf der Tiere zur Folge hat. Zum anderen unterliegt auch die Futtermittelgrundlage einem ständigen Wandel, z. B. aufgrund von pflanzenzüchterischen Maßnahmen oder infolge veränderter Standort-, Anbau- und Umweltbedingungen. Nebenprodukte aus der Verarbeitung von Lebensmitteln (z. B. Kleien oder Futtermehle), die einen erheblichen Teil der Futtergrundlage ausmachen, verändern sich nicht zuletzt durch Fortschritte in technologischen Verarbeitungsprozessen. Hinzu kommt, dass bestimmte Futtermittel seitens des Handels und der Verbraucher – unabhängig von objektiven Einschätzungen zur Futtermittelsicherheit – nicht oder in abnehmendem Maß nachgefragt werden und folglich durch Alternativen ersetzt werden müssen, derzeit z. B. importierte Sojaschrote. Auch im Bereich der Futtermittelzusatzstoffe wie Enzyme oder Konservierungsstoffe werden ständig neue Produkte entwickelt, deren Wirksamkeit untersucht werden muss. Die Herausforderungen, die sich aus dieser Dynamik in den beiden Segmenten und den zugrundeliegenden Prozessen ergeben, sind die zentralen Arbeitsgebiete der Tierernährungsforschung. Sie reichen von Themen der angewandten Tierfütterung über die Ernährungsphysiologie bis hin zu grundlegenden biologischen Aspekten des Stoffwechsels.

Die Forschung im Bereich der Tierernährung ist gemäß den zuvor skizzierten Rahmenbedingungen divers und muss mit den verschiedenen Tierarten und Nutzungsrichtungen, nicht zuletzt in Abhängigkeit vom Alter des Tieres, erfolgen. Übergreifend können die Arbeiten in die folgenden **Themenbereiche** gruppiert werden:

- Untersuchungen zur Entwicklung des Tieres, seiner Körperzusammensetzung, seines Verhaltens und Wohlbefindens, sowie seiner Verdauungs- und Stoffwechselprozesse unter Beachtung von tier- und umweltabhängigen Einflussgrößen;

- Quantifizierung des Transfers von erwünschten und unerwünschten Stoffen sowie von Erregern in das Tier, dessen Produkte und dessen Ausscheidungen;

- Ermittlung des Bedarfs und Ableitung von Versorgungsempfehlungen zur Sicherstellung der Gesundheit und Leistung verschiedener Tierarten und Nutzungsrichtungen;

- Untersuchungen zu den Konsequenzen von Stoffwechselstörungen, Erkrankungen oder Fehlernährungen sowie zur Entwicklung von vorbeugenden Maßnahmen;

- Bewertung neuer und veränderter Futtermittel und Futtermittelzusatzstoffe sowie Weiterentwicklung des methodischen Repertoires, einschließlich Entwicklung von Ersatzmethoden für Tierversuche.

Für die Beantwortung vieler Fragestellungen innerhalb des zuvor skizzierten Aufgabenspektrums ist die Durchführung von Tierversuchen unerlässlich. Ersatzmethoden, die ohne oder mit erheblich reduziertem Einsatz von Tieren auskommen, werden ebenfalls durchgeführt und präferiert, falls sie eine ausreichend gute Aussagekraft und Belastbarkeit der Informationen ermöglichen. Für bestimmte Fragestellungen gibt es bisher keine Ersatzmethoden, wie z. B. den Transfer von Futtermittelinhaltsstoffen in Produkte. Ein wichtiges Anliegen der Tierernährungsforschung ist es, Ersatzmethoden für Tierversuche weiterzuentwickeln und zu validieren, um dauerhaft eine Reduzierung von Tierversuchen zu erreichen. Grundsätzlich werden Tierversuche in der Tierernährungsforschung nur durchgeführt, wenn es für die Beantwortung der jeweiligen konkreten Fragestellung unerlässlich ist. Allerdings sind Tierversuche zur Entwicklung und zur Kalibrierung von Ersatzmethoden unverzichtbar.

2. Methoden und Techniken in Versuchen der Tierernährungsforschung

Tierversuche, die im Rahmen futtermittelkundlicher oder ernährungsphysiologischer Fragestellungen durchgeführt werden, befassen sich mit den Futtermitteln und der Fütterung sowie ihren Effekten auf das Tier, das Produkt und die Umwelt. Sie erstrecken sich auf vielfältigste Methoden und Techniken, deren Auswahl von der spezifischen Fragestellung abhängt. Grundsätzlich werden solche Vorgehensweisen ausgewählt oder entwickelt, bei deren Anwendung eine sichere und belastbare Antwort auf die Fragestellung erwartet werden kann. Die verschiedenen Versuchsansätze lassen sich zu fünf Blöcken gruppieren, die nachfolgend skizziert werden. In konkreten Versuchsvorhaben können auch Methoden aus zwei oder mehreren dieser Blöcke kombiniert werden.

a. Fütterungsversuche

In Fütterungsversuchen wird untersucht, wie sich unterschiedliche Futtermittel, Zusatzstoffe oder Techniken der Futtermittelbearbeitung auf die Futteraufnahme der Tiere, auf die Tier- und Organgesundheit, auf Leistungskriterien wie Wachstum, Milch- und Eibildung, auf die Beschaffenheit der gewonnenen Produkte oder weitere Kriterien wie z. B. das Tierverhalten auswirken. Diese Versuche erfordern gegebenenfalls besondere Haltungsbedingungen oder auch Eingriffe am Tier (siehe Punkt e.). Je nach Fragestellung werden Informationen, Befunde und Daten auch erst nach der Schlachtung von Tieren gewonnen oder es werden Tiere zur Untersuchung von Organen und Gewebe getötet, um Einflüsse auf die Gesundheit oder den Transfer von Stoffen und Erregern aufzudecken.

b. Verdaulichkeitsversuche

Verdaulichkeitsversuche sind das Fundament einer jeden rationalen Futtermittelbewertung. In Verdaulichkeitsversuchen wird ermittelt, zu welchen Anteilen im Futter enthaltene Stoffe von den Tieren mit dem Kot wieder ausgeschieden werden, um aus der Differenz zur Aufnahme den Einstrom in den Stoffwechsel des Tieres zu erfassen. Die Messung der Verdaulichkeit nach Referenzverfahren setzt voraus, dass Futter- und Kotmengen bei jedem einzelnen Tier **absolut verlustfrei** erfasst werden. Hierzu sind geeignete Haltungsformen nötig, in denen der Kot aufgefangen werden kann. In diesen Haltungsformen sind die Bewegungsfreiheit der Tiere und der Kontakt untereinander gegebenenfalls erheblich eingeschränkt. Unter Umständen kann, unter Inkaufnahme von Fehlermöglichkeiten, bei bestimmten Tierarten die vollständige Kotsammlung auch mit Beuteln erfolgen, die unter Verwendung von Hilfsmitteln am Tier befestigt werden. Das Tier hat dann mehr Bewegungs- und Kontaktmöglichkeiten, aber eine gewisse Einschränkung der Bewegung bleibt dennoch bestehen. Ein Verdaulichkeitsversuch kann auch unter Verwendung spezieller Marker durchgeführt werden, wobei sich die Kotsammlung auf Stichproben begrenzt; dabei entfällt also die Notwendigkeit einer verlustfreien Sammlung des Kotes. Allerdings ist die Genauigkeit der Datenerfassung bei Einsatz von Markern geringer als bei verlustfreier Kotsammlung, so dass eventuell eine größere Anzahl von Versuchstieren erforderlich wird. Die Eignung eines Markers kann wiederum nur in Versuchen mit verlustfreier Sammlung von Futter- und Kotmengen beurteilt werden.

Mit der Erfassung und Untersuchung des Kotes allein können Umsetzungen in einzelnen Abschnitten des Verdauungstraktes (z. B. Pansen oder Dünndarm) aber nicht näher erfasst werden. Studien in einzelnen Abschnitten des Verdauungstraktes sind jedoch für viele Fragestellungen der Tierernährung sehr bedeutsam oder gar entscheidend, z. B. zur Bedeutung des Pansens für die Tiergesundheit. Durch operative Eingriffe (siehe Punkt e.) wird hierzu ein Zugang in dem betreffenden Abschnitt des Verdauungstraktes geschaffen („Fistulierung“), über den während des Versuches eine regelmäßige Entnahme oder Eingabe von Material aus dem bzw. in den Verdauungstrakt möglich ist. Besondere Bedeutung erlangten sie im Zusammenhang mit der Entwicklung von in vitro-Simulationstechniken (z. B. Pansen der Wiederkäuer oder Blinddarm der Pferde). Hierbei werden die Stoffumsetzungen nicht im Tier gemessen, sondern in Proben, die aus dem Verdauungstrakt entnommen wurden, und welche anschließend unter definierten Bedingungen außerhalb des Tieres (in vitro) entsprechend der Fragestellung weiterverarbeitet werden. Dadurch können auch Kenntnisse zu Umsetzungen in bestimmten Teilen des Verdauungstraktes gewonnen werden. Es gibt zudem Fragestellungen zu Verdauungsprozessen in einzelnen Abschnitten des Magen-Darm-Trakts, bei denen Eingriffe am Tier vermieden werden, indem Material aus dem Verdauungstrakt unmittelbar nach der Schlachtung der Tiere entnommen wird.

c. Bilanzversuche

Fragestellungen, die in Ergänzung zu Vorgängen im Verdauungstrakt auch den Stoffwechsel jenseits der Darmwand betreffen, erfordern sogenannte Bilanzversuche, in denen zusätzlich zu den Ausscheidungen über den Kot auch die Abgabe von Stoffen über den Harn gemessen wird. Die Haltungseinrichtung muss hierbei eine verlustfreie und getrennte Sammlung von Kot und Harn ermöglichen und erfordert daher eine erhebliche Einschränkung der Bewegungsmöglichkeiten des Tieres. Marker, die ähnlich genau wie die Marker im Kot eine Ermittlung der Harnmenge ermöglichen, sind bislang nicht verfügbar. In Bilanzversuchen müssen gegebenenfalls auch die Produkte (z. B. Milch oder Eier) verlustfrei und für jedes Einzeltier getrennt täglich erfasst werden. Der Stoffansatz im Körper kann aus den Daten einer Bilanz berechnet werden, sofern keine gasförmige Abgabe des Stoffes auftritt. Alternativ kann der Stoffansatz auch über eine vergleichende Ganzkörperanalyse ermittelt werden, bei der verschiedene Tiere nach definierter Fütterung in zuvor festgelegten Zeitabständen geschlachtet werden. In solchen Studien lassen sich auch der Anteil unerwünschter Stoffe und ihr Transfer in den Körper oder in einzelne Organe ermitteln.

d. Respirationsversuche

Respirationsversuche stellen eine Erweiterung der Bilanzversuche dar, in denen zusätzlich der Verbrauch von Sauerstoff und die Abgabe von Gasen (Kohlenstoffdioxid und Methan) gemessen werden. Diese Gaswechselmessungen sind insbesondere für die Ermittlung von Veränderungen in der Körperzusammensetzung und des Energieansatzes der Tiere bedeutsam. Diese Informationen sind die unverzichtbare Grundlage aller international gebräuchlichen Systeme zur energetischen Bewertung von Futtermitteln. Außerdem ermöglicht die Quantifizierung insbesondere der Methanabgabe eine Beurteilung von Auswirkungen der Tierhaltung auf die Umwelt. Die verlustfreie Messung der Gasmengen übersteigt die Anforderungen der zuvor genannten Bilanzversuche, weil die Volumina und die Zusammensetzung der eingeatmeten und abgegebenen Gase fortlaufend bestimmt werden müssen. Im Referenzverfahren erfolgt dies in geschlossenen, gasdichten Räumen, in denen die Zu- und Abluftströme verlustfrei erfasst werden. Über diese aufwändige Technik verfügen nur wenige Forschungseinrichtungen weltweit. Es kommen daher – insbesondere zur Ermittlung der Methanabgabe von Rindern – Verfahren zum Einsatz, die eine Schätzung der Methanmenge mit größeren Tierzahlen ohne die Notwendigkeit einer Fixierung ermöglichen (z. B. Nahinfrarotabsorptionstechniken oder Tracergase). Diese Schätzverfahren müssen zuvor oder parallel an Ergebnissen aus Respirationsversuchen kalibriert werden, sind aber dennoch mit teilweise erheblichen Ungenauigkeiten verbunden.

e. Weitere Versuchstechniken

Weitere Techniken, häufig in Kombination mit den unter a. bis d. genannten Versuchstechniken eingesetzt, sind die Entnahme von Blutproben durch eine Venenpunktion und das gesonderte Ableiten und Auffangen des Harns. In diesem Zusammenhang verdienen auch Versuchsansätze Erwähnung, in denen mit der Ausschaltung eines Organs oder der Ableitung eines Sekrets (z. B. von Speichel) die ganz spezifischen Funktionen dieses einen Organs oder eines Sekrets für die Verdauung insgesamt geprüft werden. Des Weiteren können zur Untersuchung von z. B. allergischen Reaktionen oder des Stoffwechsels Gewebe- und Organproben am lebenden Tier entnommen werden (Biopsien von Leber, Fettgewebe und Muskulatur unter Anästhesie bzw. in Narkose). Schließlich seien auch Versuche genannt, in denen die Bedeutung des Futters oder von Futterzusatzstoffen für den Ablauf von Infektionen in Tierbeständen geprüft werden. Diese Versuche sind insbesondere bei solchen Infektionen bedeutsam, die vom Tier auf den Menschen übertragen werden können (sogenannte Zoonosen, z. B. Salmonellose). Um die Infektionswege und die Wirksamkeit von Maßnahmen zur Einschränkung der Übertragung zu erforschen, werden die Erreger einzelnen Tieren verabreicht, so dass diese als „Ausscheider“ in einer Tiergruppe fungieren. Solche Versuche sind von herausragender Bedeutung, wenn geeignete Präventionsmaßnahmen seitens der Fütterung entwickelt werden, mit denen die Lebensmittelsicherheit verbessert werden kann (Vermeidung einer Infektion von Menschen).

3. Einschätzung der Methoden und Ansatzpunkte zur Verminderung von Anzahl und Belastung der Versuchstiere

Tierversuche sind sehr aufwändig und beginnen daher mit sorgfältiger Planung unter Beachtung des nationalen und europäischen Rechtsrahmens. Die Planung beinhaltet die Klärung der Frage, ob der Versuch wissenschaftlich begründet und notwendig ist, und ob Ersatzmethoden zur Verfügung stehen. Versuche mit Tieren werden nur dann in Erwägung gezogen, wenn geeignete Ersatzmethoden nicht zur Verfügung stehen und der Tierversuch somit unerlässlich ist.

Ersatzmethoden in der Tierernährungsforschung reichen von reinen Analysenverfahren im Labor über in vitro-Versuche und Zellkultur- oder Gewebestudien bis zu Computersimulationen. Mit diesen Ersatzmethoden sollen Abläufe in Organen, Tieren oder Tierbeständen bestmöglich abgebildet werden. Sie sollen in der Arbeitsroutine einsetzbar sein und müssen hinreichend genau sein. Daher können sie nur anhand von Daten aus Tierversuchen generiert, abgeleitet und regelmäßig validiert werden. Die Entwicklung und Verbesserung von Ersatzmethoden ist somit Bestandteil wissenschaftlichen Arbeitens mit ernährungsphysiologischem oder futtermittelkundlichem Hintergrund. Ersatzmethoden können die komplexe biologische Realität eines ganzen Organismus allerdings nur unvollständig abbilden und sind stets mit Unsicherheiten und Fehlermöglichkeiten behaftet. Bei der Planung von Versuchsvorhaben ist in jedem Einzelfall zu entscheiden, ob der Schätzfehler einer Methode für die spezifische Versuchsfrage akzeptabel ist und eine Ersatzmethode folglich verwendet werden kann, oder ob die angestrebte Genauigkeit einen Tierversuch zwingend erforderlich macht.

Wird ein Tierversuch für unerlässlich befunden und geplant, ist es das Ziel, die Anzahl der Versuchstiere auf ein Minimum zu reduzieren. Hierzu wird eine biometrische Planung vorgenommen, in die Vorinformationen zur Variation der Messwerte und Erwartungswerte zur Höhe eines Versuchseffektes einfließen. Die Tieranzahl wird dabei so bemessen, dass ein Versuchseffekt – sofern er vorhanden ist – im Versuch auch tatsächlich nachgewiesen werden kann. Für bestimmte Tierversuche wie z. B. Verdaulichkeitsbestimmungen gibt es zudem von wissenschaftlichen Gremien entwickelte Leitlinien zur Planung und Durchführung, die zur Orientierung auch Mindestwerte für die Anzahl von Versuchstieren festlegen.

In Verdaulichkeits-, Bilanz- und Respirationsversuchen stellt die Einschränkung der arttypischen Bewegungs- und Kontaktmöglichkeiten einen Stress dar. Die Stressbelastung kann in Abwägung aller Argumente akzeptiert werden, wenn es zur Beantwortung der Fragestellung keine Alternative gibt. In vielen Fällen werden in Versuchen mit einer kleinen Zahl von Tieren Erkenntnisse gewonnen, die für große Populationen von Nutztieren, den Menschen oder die Umwelt erhebliche Vorteile ergeben. Die Dauer der Einschränkung wird dabei auf das Mindestmaß begrenzt. Es gibt Leitlinien zur Durchführung von Verdaulichkeitsversuchen, die unter Beachtung der Tierart festlegen, wie lange die verlustfreie Sammlung des Kotes dauern muss, damit eine repräsentative Aussage zur abgegebenen Kotmenge erreicht wird. So wird von der Gesellschaft für Ernährungsphysiologie derzeit eine Dauer von fünf Tagen für Schweine und von mindestens sieben Tagen für Schafe empfohlen, wobei laufende Projekte die Frage beantworten sollen, ob diese Zeiträume verkürzt werden können. Reaktionen des Tieres auf Stressoren können erheblich reduziert werden, wenn Tiere nicht einzeln, sondern zu zweit oder in kleinen Gruppen gehalten werden, oder zumindest ein Sichtkontakt sichergestellt ist. Eine der wichtigsten Maßnahmen ist dabei die langsame und allmähliche Gewöhnung der Tiere an die besondere Haltung. Tierbetreuer sind gehalten, viel Zeit in den Stallanlagen mit Kontakten zu den Versuchstieren zu verbringen und durch intensive Betreuung für eine entsprechende Beschäftigung der Tiere zu sorgen.

Bestimmte Fragestellungen der Tierernährungsforschung erfordern den zuvor erläuterten operativen Zugang zum Verdauungstrakt, um am lebenden Tier gezielt Magen- oder Darminhalt entnehmen zu können. Diese operativen Eingriffe erfolgen unter entsprechender Anästhesie oder Narkose, erfordern für eine kurze Zeit auch eine medikamentöse Schmerzausschaltung und unterliegen generell besonderen Prüfungen hinsichtlich ihrer Vertretbarkeit. Nach entsprechender Abheilung können fistulierte Tiere in Abhängigkeit von der Tierart bei entsprechender Pflege über Monate bis viele Jahre als „Spendertiere“ dienen, so dass entsprechende *in vitro*-Verfahren wie z. B. der Hohenheimer Futterwerttest ermöglicht werden, die ganz erheblich zur Reduktion von Tierversuchen beitragen.

Die Frage, ob die Dauer der Bewegungseinschränkung insbesondere in Bilanzversuchen ohne Verlust an Aussagegenauigkeit weiter reduziert werden kann, gegebenenfalls auch durch eine Erhöhung der Tierzahl, ist Gegenstand aktueller Forschungsprojekte. In diesem Zusammenhang werden interdisziplinäre Initiativen begrüßt, in denen Verhaltensforscher Indikatoren entwickeln, die eine stärker objektivierte Beurteilung des Empfindens von Stress und Belastung durch das Tier bei Variation von Haltungsbedingungen und -dauer, einschließlich einer wiederholten Nutzung derselben Tiere, ermöglichen.

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