Proceedings of the Society of Nutrition Physiology

Berichte der Gesellschaft für Ernährungsphysiologie

Band 27, 2018

DLG Verlag

Proceedings of the *Society of Nutrition Physiology*

Berichte der Gesellschaft für Ernährungsphysiologie

Editor: Society of Nutrition Physiology
Herausgeber: Gesellschaft für Ernährungsphysiologie

Volume 27, 2018
Band 27, 2018

72nd Conference 13th–15th March 2018 in Göttingen
72. Tagung vom 13.–15.03.2018 in Göttingen

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

Deutsche Bibliothek - CIP Einheitsaufnahme

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

ISBN 978-3-7690-4111-8

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

Conference abstracts were peer-reviewed.

Members of the reviewer panel were:

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

W. Windisch
Chairman

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Linking post-absorptive metabolism of amino acids and ration formulation in dairy cows

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INTRODUCTION

To the continued challenge of making dairy farming more cost effective is now added the pressure of reducing environmental pollution. Indeed, excretion of N, especially in the urine, is a potential source of water (e.g. nitrates) and air pollution (e.g. N_2O, a greenhouse gas, or as small particulate polluting aerosols; National Research Council (NRC) 2003). Increased cost effectiveness and decreased pollution can be achieved through a lower input of dietary protein, provided productivity is not compromised. To accomplish this without compromising productivity requires that feeds are better balanced to match supply with requirement (rqt) of proteins and amino acids (**AA**).

It is acknowledged that improving the formulation of dairy rations requires accurate estimation of both supply and rqt of metabolizable protein (**MP**), far beyond the sole estimation of crude protein (**CP**). A further step involves moving the estimations of supply and rqt from MP to individual essential AA (**EAA**). Predictions of duodenal flows of proteins or EAA from different feeding systems agree quite well with measured values (Pacheco *et al.*, 2012), although fine-tuning of these models might still be needed to improve predictions with multiple and variable feed ingredients. However, the intensive and regular efforts to improve the predictions of supply over the past decades have not been matched by similar efforts to improve the estimations of rqt. In recent years, however, many Europeans feeding systems have been revisited (e.g. NorFor, 2011; Van Duinkerken *et al.*, 2011; Sauvant *et al.*, 2015) and have included the latest knowledge in their estimation of rqt. The assumed linear relationship between supply and output arising from the use of a fixed efficiency has been progressively changed to a variable efficiency linked to supply of both protein and energy or dry matter intake (**DMI**). We will overview together how the post-absorptive metabolism of AA supports these changes adopted for MP rqt and other changes to come to balance dairy rations for individual AA.

POST-ABSORPTIVE METABOLISM OF AMINO ACIDS

In dairy cows, metabolism of AA has been mainly studied across the mammary gland (an obvious target for research and production) and to a more limited extent across the splanchnic tissues, the latter including the portal-drained viscera (**PDV**: gastro-intestinal tract, spleen, pancreas and associated mesenteric fat) and the liver.

Net absorption and utilization of AA across the portal-drained viscera

Once the proteins are hydrolysed, the individual AA are absorbed across the intestinal epithelium, part of them reaching the mesenteric and then the portal blood circulation. The net portal absorption (**NPA**) of AA is calculated as the flow of AA in the portal vein minus the flow from arterial supply: as such, NPA is the result of two opposite actions: 1) the absorption of AA from digested protein in the small intestine which increases the NPA of AA and 2) utilization of AA by the whole PDV which decreases the NPA. Although the driving force of the flow of protein digested, and subsequently NPA of AA, is N intake, dietary parameters related to energy such as NDF intake were significant covariates in the relationship between N intake and NPA of AA (Martineau *et al.*, 2011). Indeed, over a wide range of diets (75 experiments, 174 diets) in sheep and in cattle, the energy available in the diet increased NPA of AA whereas NDF intake had the inverse effect, probably partly in relation to their respective effect on microbial protein synthesis. In addition, within individual studies, NPA of AA was not only related to the CP intake. For example, an increased MP supply, resulting from increased rumen undegraded protein supply, increased NPA of AA by 21%, despite the fact that CP intake was similar between diets (Blouin *et al.*, 2002). So, clearly, N (or CP) intake is not the only factor to consider for an adequate estimation of the supply of AA.

As mentioned previously, the NPA of AA is also affected by the rate of AA used by the whole PDV. Measurements of AA utilization across the PDV includes many challenges with 2 sources of supply (intestinal absorption and arterial supply), and inclusion of endogenous AA in the duodenal, ileal and fecal digesta flows. First attempts to determine the net utilization of AA across the PDV were made in sheep comparing the

small intestinal disappearance with NPA: the recovery in the portal vein of the amount that had disappeared from the small intestine ranged from 19% for histidine to 69% for lysine, suggesting a huge net utilization of AA by the gut (Tagari and Bergman, 1978). Later, data in sheep (MacRae *et al.*, 1997a) and in dairy cows (Berthiaume *et al.*, 2001) reported higher recoveries than in this initial study, ranging from 43% (threonine) to 95% (histidine). However, does this proportion really represent what is being lost through passage across the gut? We have to keep in mind that part of the small intestinal disappearance is not a net supply because the endogenous proteins secreted prior to the duodenum that are digested and reabsorbed do not contribute to the net supply. Therefore, even without any net utilization of AA by the gut tissue, portal absorption will always be less than apparent small intestinal disappearance. Indeed, using ^{13}C-labelled AA, MacRae *et al.* (1997b) reported that besides phenylalanine, more than 80% of the EAA used by the PDV were from arterial origin.

The real losses of AA across the gut are oxidation and endogenous secretions that are not reabsorbed and therefore excreted in the feces. There is very limited data in ruminants on endogenous protein secretions and gut oxidation, both processes being very challenging to measure directly. Oxidation of EAA across the PDV is, however, known to occur as leucine oxidation across the PDV has been reported dairy cows (Lapierre *et al.*, 2002), whereas leucine and methionine oxidation has also been measured in sheep (Lobley *et al.*, 2003). In addition to oxidation, the loss of EAA across the PDV also occurs through the secretion of endogenous proteins which are not reabsorbed across their transit in the small intestine: they are recovered as undigested endogenous proteins flowing at the ileum, but usually measured in the feces. These would be the best estimations of what is usually referred to as the metabolic fecal protein: we will keep the nomenclature of "metabolic fecal protein" but should remember that the true loss should be measured as the endogenous proteins flowing at the ileum. Based on the measurement of endogenous protein flow across the gut by Ouellet *et al.* (2002, 2007, and 2010), and Sandek *et al.* (2001) combined to the meta-analysis of Marini *et al.* (2008), the metabolic fecal protein has been estimated to (g of true protein/d) = [(8.5 + 0.1 × $NDF_{\%DM}$) × DMI], with an AA composition from a composite value of ruminal and abomasal isolates in cattle and ileal endogenous secretions in pigs (Lapierre *et al.*, 2016a). In dairy cows, comparison of the estimations of digestive flow of AA and measured NPA agreed well with substantial losses across the PDV of the branched-chain AA (BCAA: isoleucine, leucine and valine), probably via oxidation, and of threonine, most likely due to its high concentration in endogenous secretions (Lapierre *et al.*, 2006; Pacheco *et al.*, 2006). Overall, despite the fact that exact amounts and regulatory mechanisms are still unknown, there is enough evidence to consider significant net utilization of all EAA by PDV tissues: all EAA are needed for the synthesis of the endogenous proteins which will be partly undigested and secreted in the feces; furthermore, some EAA, but not all, are also oxidized by the PDV.

Net utilization of AA across the liver

Due to the anatomical location of the liver, information on AA metabolism across the liver is easier to collect than across the gut. From a database of studies where N transfers across the splanchnic tissues had been studied (22 treatments), the liver removed, on average, 45% of total AA being absorbed (AA being measured individually or as alpha-amino-N; Lapierre *et al.*, 2005). We have to be very cautious, however, because this average number cannot be applied to each individual AA. This removal encompasses all AA, EAA and non-EAA, but the liver removes AA for different purposes. Among other functions, it has the important role of avoiding hyperaminoacidaemia and therefore extracts AA in excess through urea synthesis; it also uses AA for the synthesis of proteins exported to the plasma (Raggio *et al.*, 2007); it catabolizes some AA, mainly non-EAA, for the synthesis of glucose. These different roles of the liver already suggest variable hepatic removal of the different AA.

To acknowledge the fate of individual AA across the liver, another database, where the net splanchnic flux of individual AA had been measured, was used (12 treatment means; Lapierre *et al.*, 2012). Two families of EAA were quite distinctive in their behaviour across the splanchnic tissues and related well with the groups described by Mepham (1982) according to their metabolism across the mammary gland. Amino acids from Group 1, for which mammary uptake is about equal to secretion into milk protein, are substantially removed by the liver and post-liver supply is approximately equal to mammary uptake. Histidine, methionine, phenylalanine plus tyrosine, and tryptophan are included in this group. On average, hepatic removal relative to NPA varied from 31% for methionine to 51% for phenylalanine (Lapierre *et al*., 2012). In contrast, AA from Group 2, already identified as those for which mammary uptake exceeds AA in milk protein output, are, on a net

basis, barely removed by the liver, even often with a slightly positive net flux, and with a post-liver supply higher than mammary uptake. Group 2 consists of the BCAA and lysine. Therefore, despite the fact that the liver is the major site of ureagenesis, not all of the EAA in excess are, on a net basis, extracted by the liver. They can be deaminated elsewhere in the body and the N returned to the liver through N-shuttles like alanine or glutamine prior to excretion of excess N as urea. The distribution of enzymes responsible for AA catabolism is directly linked with the two groups described above. For Group 1 AA, degradative enzymes are predominantly restricted to the liver whereas for Group 2 AA, the enzymes responsible for catabolism are widely distributed across tissues, including liver, muscle, fat, gut and mammary gland (Lobley and Lapierre, 2003).

It has also been suggested that liver removal of AA is related to NPA (Lescoat *et al.*, 1996). In practice, increased NPA of AA is usually associated with larger plasma concentrations and in such situations, we cannot dissociate if either or both are regulating factors of hepatic removal. Dissociation between these two parameters has been established under physiological conditions, with cows before and after initiation of lactation (Doepel *et al.*, 2009): lactation increased intake and therefore NPA of AA, whereas the high demand to support milk protein secretion led to reduced concentrations of AA. In such a scenario, net liver removal of EAA is not driven by NPA. Indeed, it has been suggested that liver removal may be better correlated with total liver inflow rather than with NPA (Hanigan, 2005; Lapierre *et al.*, 2005), because total inflow integrates both NPA and arterial supply, the latter reflecting the utilization of EAA by peripheral tissues. Such a concept implies that hepatic extraction is not exclusively due to first pass removal but rather that a constant fraction of the total liver inflow is extracted per pass, with the amount returned to the liver driven by peripheral tissue utilization. Indeed, NPA contributes only between 5% (valine) to 20% (methionine) of total liver inflow in dairy cows, and the liver removes only 3, 8, 9 and 3% of the total inflow for histidine, methionine, phenylalanine and threonine respectively, whereas extraction of the BCAA and lysine are less than 1% of the inflow (Lapierre *et al*., 2005). With this background, peripheral tissues have the opportunity over a short window of time to use absorbed AA before they are finally catabolized by the liver after a few passes across the splanchnic bed.

Net uptake of AA across the mammary gland

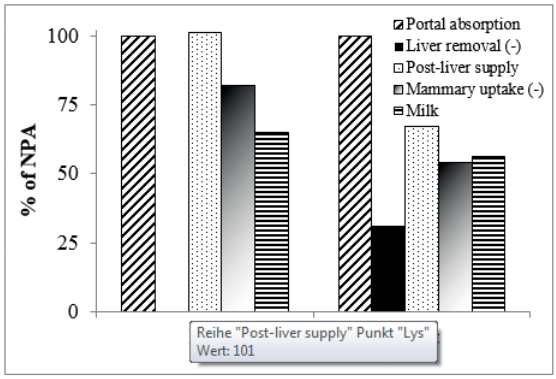
Continuing their journey post-liver, the AA flow into blood circulation to feed peripheral tissues. In multiparous lactating dairy cows, the primary net user of absorbed AA is the mammary gland. For those AA catabolized predominantly within the liver, net splanchnic flux appears to be almost totally captured by the mammary gland and secreted quantitatively in milk protein. Indeed, in dairy cows, the ratio of milk output to net post-liver supply ($n = 12$) averaged 0.99 ± 0.12, 0.97 ± 0.10, and 0.96 ± 0.12 for histidine, methionine and phenylalanine + tyrosine, respectively (Lapierre *et al.*, 2012). The mammary uptake:output ratio in studies where samples have been analysed individually ($n = 13$) averaged 1.05 ± 0.05, 1.01 ± 0.04 for histidine and methionine, respectively: phenylalanine and tyrosine have been used as markers to estimate mammary plasma flow. Methionine, phenylalanine + tyrosine, and tryptophan have been grouped in Group I by Mepham (1982) for their stoichiometric transfer from blood to milk protein: histidine was later added to this group (Lapierre *et al*., 2012). The capture by the mammary gland of these AA is therefore critical for milk protein synthesis and mechanisms have evolved to maintain uptake even when supply is limited. For example, when histidine supply was limited in dairy goats, the mammary gland blood flow increased by 33% and the capacity to remove plasma histidine increased by 43 fold, to reduce the impact of the lowered arterial supply (Bequette *et al.*, 2000).

On the other hand, for Group 2 AA (BCAA and lysine), post-liver supply usually exceeds mammary uptake which itself also exceeds output in milk protein. Leucine is known to be oxidized within the mammary gland (Bequette *et al.*, 1996; Raggio *et al.*, 2006), as is lysine (Mabjeesh *et al.*, 2000). For leucine at least, this oxidation is probably not obligatory as a decrease in protein intake decreased leucine catabolism at the whole body and mammary level, with limited penalty on milk protein production (Bequette *et al*., 1996; Raggio *et al*., 2006). Similarly, the ratio of mammary uptake to milk output for the BCAA and lysine decreases when supply of these AA decreases (Lapierre *et al*., 2012), in agreement with a reduced mammary oxidation at lower MP supply (Raggio *et al.*, 2006).

Why does the mammary gland extract AA of Group 2 in excess of its needs for milk protein synthesis? As the mammary gland uptake of non-EAA is less than output in milk protein (e.g. Guinard and Rulquin, 1994; Doepel and Lapierre, 2010) then obviously de novo synthesis must occur and this requires sources of N. Leucine and

the other BCAA have proved to be excellent transamination sources in other tissues and support synthesis of non-EAA. The extraction in excess of the BCAA would provide such a N source. Similarly, the N of lysine taken up in excess by the mammary gland was shown to support synthesis of non-EAA, particularly glutamate and aspartate (Lapierre *et al.*, 2009). Therefore, along with the BCAA, the catabolism of lysine has been 'shifted' metabolically from the liver to the mammary gland in order to support the necessary non-EAA synthesis required to maintain milk protein output. It has also been recently proposed that the excess mammary uptake of AA from Group 2 could be used to generate ATP within the mammary gland to support the increased protein synthesis (Lemosquet *et al.*, 2010). Figure 1 summarizes the relation between the net flux of lysine and methionine across the PDV, the liver, the mammary gland and milk protein secretion (Lapierre *et al*., 2012)

Figure 1. Net flows of two essential amino acids representative of Group 1 (methionine) and Group 2 (lysine) in dairy cows, expressed as a percentage of net portal absorption (NPA; from Lapierre *et al.,* 2012)



FROM METABOLISM TO RATION FORMULATION

Based on the observed metabolism of individual AA, some paradigms included in feeding systems have been either changed or fine-tuned.

Endogenous protein flow along the gastro-intestinal tract

A better knowledge of the fate and quantification of the endogenous protein flow across the gut has been used to improve how endogenous proteins are included into feeding systems.

Endogenous duodenal flow. Acknowledging that most part of the endogenous duodenal flow is originating from arterial supply, i.e. from previously absorbed AA, the endogenous duodenal flow should not be considered as a net supply to the animal. It however needs to be quantified to compare estimates and observed duodenal flow, these being the sum of undegradable dietary, microbial and endogenous proteins. Also, as part of the endogenous duodenal protein flow is digested and reabsorbed during transit across the small intestine, it should also not considered as a requirement. Indeed, the real loss is only the amount that is not re-absorbed, flowing at the ileum and secreted in the feces, which is in fact the metabolic fecal protein discussed above. The endogenous duodenal flow has been estimated as (g CP/d) = 96.1 + 7.54 × $DMI_{kg/d}$ (Lapierre *et al.*, 2016b). Norfor (2011) estimates the endogenous duodenal flow of CP (g/d) as rumen outflow of organic matter × 0.03 whereas it is estimated as 14.2 g CP/kg DMI in the new French system (Sauvant and Nozière, 2016).

Metabolic fecal requirement. Once correctly acknowledged that the part of metabolic fecal protein excretion creating a demand on AA is only the fraction synthesized from AA and not the portion synthesized from urea, estimations of metabolic fecal protein have been reduced compared to Swanson (1977) estimations. This allowed an efficiency factor to be assigned to this component, which was not the case in NRC (2001) or CNCPS (Fox *et al.*, 2004). It also includes a small portion lost in the hindgut and although still referred to as metabolic "fecal" protein it rather refers to flow of the endogenous protein flowing at the ileum. Proposed estimations (g true protein/d) for the net rqt were either based on meta-analysis (e.g. [0.5 × (5.7 + 0.074 × undigested organic $matter_{g/kgDMI}$) × DMI], (Sauvant *et al.*, 2015) or on integrations of more limited but observed data (e.g. [(8.5 + 0.1 × $NDF_{\%diet}$) × $DMI_{kg/d}$]; Lapierre *et al*., 2016a).

Efficiency

Combined efficiency. Traditionally, an efficiency is assigned to each of the net protein or AA rqt to determine the total protein or AA rqt. For example, in NRC (2001), to determine protein rqt an efficiency of 0.67 is assigned to endogenous urinary, scurf, and milk, whereas an efficiency of 1 is attached to metabolic fecal protein and 0.33 to pregnancy. The CNCPS feeding system was initially attributing different efficiency to individual AA, depending if used for maintenance or productive function as pregnancy or milk: for example, for lysine vs. methionine respectively, the efficiency for maintenance, pregnancy and milk were 0.85, 0.53 and 0.82 vs. 0.85, 0.35 and 1.00 (Fox *et al.*, 2004).

As stated earlier, based on AA metabolism depicted in Figure 1, it appears, however, quite clearly that the site of AA catabolism is not related to the site of protein synthesis or exportation: AA of Group 1 (e.g. methionine in Figure 1) are not catabolized across the mammary gland, despite their use to support milk protein secretion. In fact, most, if not all, their catabolism occurs across the liver. Catabolism of lysine and Group 2 AA occurs in the PDV, peripheral tissues and mammary gland. Based on these observation, Lapierre *et al.* (2007) suggested to use a single combined efficiency for maintenance and lactation. The last published version of CNCPS has opted for a combined but fixed efficiency of AA utilization, varying between 57% and 76% (Van Amburgh *et al.*, 2015). Systali also has opted to use a combined variable efficiency as it was yielding a better fit of the data than a fixed efficiency for maintenance and a variable efficiency for milk (Sauvant *et al.*, 2015). NorFor (2011) opted for a fixed efficiency of 0.67 or 1.00 (depending of the component) for maintenance and a variable efficiency for milk.

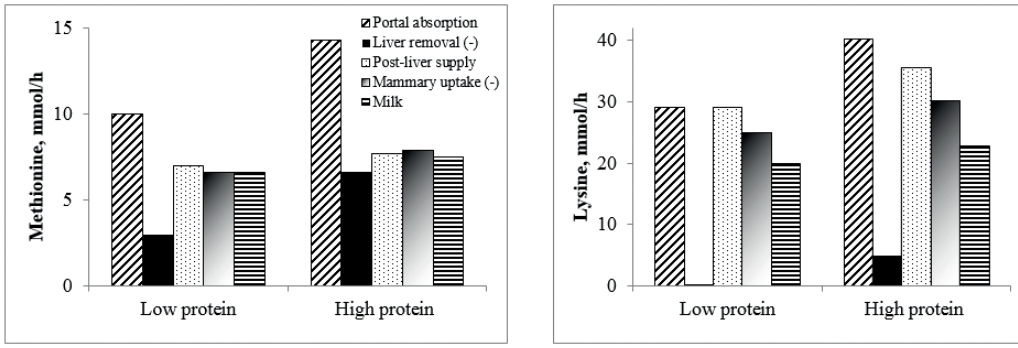
Variable efficiency. The first section has presented average net flows of EAA across the PDV, the liver and the mammary gland. A closer look at EAA net flows across the tissues in relation to their availability would help to determine if their removal is fixed or altered with supply. Data on losses of AA across the PDV in relation with supply are very scarce in dairy cows. The oxidation of leucine by the PDV increased with MP supply (Lapierre *et al*., 2002), suggesting that the PDV oxidation of leucine, and most likely, the other BCAA, increases with increased supply. For the AA removed by the liver, the liver has the dual role of removing excesses to avoid toxicity, while still providing that needed to support anabolism in peripheral tissues. How the fine-tuning of regulation between catabolism and anabolism is achieved is not known yet. It was first proposed that the liver actively controls the post-hepatic supply of AA (the resultant of NPA minus hepatic removal) and thus regulates peripheral tissue protein "gain" including milk protein secretion. Indeed, in dairy cows, when the post-hepatic supply of histidine, methionine and phenylalanine (the EAA removed to the greater extent by the liver) are compared with mammary gland and milk protein secretion, the close balance might support the theory of the liver as the controller of the amount of AA supplied to peripheral tissues. But, on further consideration, as catabolism of Group 1 AA is primarily hepatic, then the apparent perfect balance could simply be a consequence of removal of any excess. Despite a lack of final resolution as to the signals, two messages arise from these relationships. First, as plasma concentrations are always finite (as will be hepatic inflow) then liver extraction of these EAA will never decline to zero. This would explain why, in circumstances such as the beginning of lactation when intake is insufficient to meet the requirement for high milk production, there is still substantial removal of AA by the liver (Doepel *et al.,* 2009). The other message is: "The higher are the circulating concentrations, the more will be removed"!

For the AA removed by the mammary gland in excess of that needed to cover secretion into milk protein, the uptake to output ratio has also been reported to increase with an increasing dose of casein or AA infused post-ruminally (Guinard and Rulquin, 1994; Raggio *et al.*, 2004 and 2006; Doepel and Lapierre, 2010) suggesting indirectly an increased catabolism of these AA in the mammary gland. Indeed, in dairy cows, increased leucine availability increased oxidation of leucine in the mammary gland (Bequette *et al*., 1996; Raggio *et al*., 2006). Globally, it was reported in a meta-analysis that the excess uptake of the Group 2 AA increases with the protein supply (Lapierre *et al*., 2012). Similarly, increased lysine supply amplified the excess of mammary uptake of lysine relative to milk output. In parallel, the contribution of the N from lysine taken up in excess for the synthesis of non-EAA also increased with increased lysine (Lapierre et al., 2009). However, this excess of uptake of AA from Group 2 relative to milk output is not essential to maintain milk protein output (Bequette *et al*., 1996; Lapierre *et al*., 2009).

To bring all this information together, we measured, in a single study, net AA metabolism across the PDV, the liver and the mammary gland, at different protein intakes in dairy cows (Raggio *et al*., 2004). First, milk protein yield achieved by these cows was higher than predicted by NRC (2001: 802 vs. 714 g/d) at low protein intake (12.7% CP, 1922 g/d MP) and lower than predicted (902 vs. 1100 g/d) at high intake (16.6% CP, 2517 g MP/d; NRC, 2001). Second, post-liver supply of EAA was sufficient to cover milk protein yield, which suggests that utilization of body protein (i.e. negative N balance) was not the reason for the increased efficiency, except for histidine whose supply could have come from muscular dipeptides. Figure 2 shows the net flows of two AA across tissues: methionine is presented as an example of Group 1 AA and lysine as an example of Group 2 AA. We can see that the removal of methionine across the liver and of lysine across the mammary gland and peripheral tissues increased at a higher proportional rate than the increased portal absorption: this resulted in a decreased efficiency at higher protein intake. This increased removal of AA was directly linked with a more than doubled urinary-N excretion at the high compared with the low protein intake, averaging 165 and 79 g of N/d, respectively. For lysine and methionine, the ratio of milk protein output on portal absorption averaged 0.68 vs. 0.56 and 0.66 vs. 0.52 for low vs. high protein supply, respectively. If we remove estimated requirements for maintenance (NRC, 2001) to calculate the efficiency of lactation, these efficiencies of lactation averaged 0.96 vs. 0.71 for lysine and 0.95 vs. 0.66 for methionine at low vs. high protein supply respectively. Clearly, at lower protein supply, the cow has the capacity to reduce AA catabolism.

Therefore, it is clear that the efficiency of utilization of absorbed AA should not be a fixed factor, but numbers presented earlier were only estimated from one study. Can we predict the efficiency in relation with supply? In an effort to make that prediction, a database (59 trials) was built compiling all published reports where AA had been infused post-ruminally (Doepel *et al.*, 2004). Linear and non-linear models were tested to relate milk AA-protein output with AA supply (with only the control treatment relying on a predictive model to estimate digestible AA). The segmented-linear (fixed efficiency followed by a 0 response when the requirements are met, and logistic models were similar in their reliability (Doepel *et al.*, 2004). The logistic model, however, offers the possibility to estimate variable efficiency of transfer, which was decreasing as protein supply increased. These were quite close to the efficiencies estimated in the study of Raggio *et al.* (2004). The logistic model was later updated to estimate a combined efficiency where the efficiency of utilization of individual AA was expressed relative to AA supply (Lapierre *et al.*, 2007) or the combiend efficiency of utilization of MP was expressed realtive to total MP supply, once the endogenous urinary export was removed from both parameters (Moraes *et al.*, 2018). Further work, however, has integrated not only the supply of AA, but also the supply of energy to estimate the utilization of AA (Lapierre *et al.*, 2017). The efficiency was better predicted using the ratio of AA/NE_L supply, as also proposed in NorFor (2011), in the DVE/EOB system (Van Duinkerken *et al.*, 2011) whereas Sauvant *et al.* (2015) rather used the supply of AA/DMI ratio as the best predictor of the efficiency of utilization of MP. Work is currently undergoing to use both the supply of AA and of NE_L as independent variable to estimate the efficiency of utilization of MP and AA. Experimental work has also been conducted on the AA flows across the splanchnic and mammary tissues when these two parameters are altered, but compilation of the results is not finished yet.

Figure 2. Net flows of two essential amino acids representative of Group 1 (methionine) and Group 2 (lysine) across tissues in dairy cows at two protein feeding levels (from Raggio *et al.*, 2004)



CONCLUSION

Overall, a better knowledge of the transit of endogenous proteins along the digestive tract has improved the quantification of the net supply of MP and AA and of the daily amount of exported AA. In addition, knowledge of AA metabolism has suggested 1) using a combined efficiency for the protein functions (except endogenous urinary excretion) and 2) using a variable efficiency to convert these exported MP and AA into requirements. Although it was first suggested that the efficiency of MP or individual AA was related to their digestive flow, inclusion of NE_L supply, as an independent variable or either as a ratio with MP or AA supply, improves its prediction. In a whole feeding system, optimization of the efficiency of the different EAA should allow an improved estimation of the rqt and prediction of milk protein yield under known supply.

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Abstracts

1.

Effects of pancreatic exocrine insufficiency (with or without pancreatic enzyme therapy) on ileal and faecal microbiota of ileo-caecal fistulated minipigs

Effekte der exokrinen Pankreasinsuffizienz (ohne bzw. mit Enzymsubstitutionstherapie) auf das Mikrobiom von Ileumchymus sowie Faeces von ileo-caecal fistulierten Minischweinen.

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Introduction: The pancreatic exocrine insufficiency (PEI) is a disease and characterised by maldigestion and malabsorption. The reduced enzymatic digestive capacity causes a higher influx of undigested nutrients into aboral parts of gastrointestinal tract with forced bacterial fermentation (1). In former studies, using the animal model of the pancreatic duct ligated (PL) pig, marked effects of PEI (not treated with enzymes) on ileal microbiota (16sRNA) were found (2). However, no comparative investigations about effects of pancreatic enzyme therapy (PERT) on intestinal microbiota on individual basis were possible since samples were obtained at slaughter. This study aimed to investigate changes in microbiota caused by PEI comparing ileal and faecal samples and whether PERT can normalise these changes.

Methods: Ileal digesta and faeces of 7 pigs with experimentally induced PEI fitted with an ileo-caecal fistula were taken during phase of PERT and after 10 days without PERT. 5 healthy control pigs were sampled as well. All animals received exactly the same complete diet. The V1-V2 region of the 16S rRNA gene was amplified and sequenced on the MiSeq Illumina platform. Obtained sequences were merged, quality filtered and assigned a taxonomy down to the genus level using RDP"s naive Bayesian classifier. All analyses were done on the genus level on rarefied count data.

Results: Shannon diversity index (SDI) was lowest in PEI pigs without PERT for ileal as well as faecal samples. Ileal microbiota showed a high individual variation. up to factor 10 for relative abundance. When PERT was given Lactobacillus (Lb) was the most abundant genus in ileal digesta (27.5 %) while Bifidobacterium (Bif) accounted only for 5.53 %. After 10 days without PERT Bif were the most abundant species (28.8 %) and Lb accounted for 19.2 % . The increase of Bif on individual basis during phase without PERT varied between factor 1.7 and 86 with a mean of 23. Similarly for faecal samples Lb and Bif were markedly higher without PERT (see table 1). Concentration (mg/kg wet weight) of sum of short chain fatty acids was higher during phase without PERT (11470) compared to phase with PERT (6379).

Table 1: Shannon diversity index (SDI) and relative abundance of Lactobacillus (Lb) and Bifidobacterium (Bif) as well as Escherischia and Shigella (E / Sh) in ileal chyme (Ileum) and faeces of healthy control pigs and pigs with PEI (with or without PERT)

a,b: sign. effect ($p<0.05$) between controls and PL-pigs; +, #: sign. effect ($p<0.05$) of PERT in PL-pigs; comparison was always made within samples of same location (ileal chyme and faeces)

Discussion: Overall community composition did significantly differ between sites and with treatment. The results indicate that faecal samples might be a useful matrix to detect effects of PEI and PERT in general, but do not allow direct conclusions about microbiota in the small intestine (3). It is worth mentioning that Bif markedly increased in faecal samples of PL. Considering the markedly increased number of Bif caused by a higher influx of fermentable carbohydrates into the aboral parts of the gastrointestinal tract is associated with clinical symptoms it seems questionable whether Bif are still „healthy" when occurring in very high abundance.

Conclusion: PERT markedly affected microbiota of ileal digesta and faeces. During phase without PERT Bif increased and SDI was lower in microbiota of ileal digesta as well as in faeces.

1) Capurso et al. 2016, ueg journal 4(5), 697-705;
2) Mößeler et al. 2016 Proc. ESVCN, 69;
3) Suchodolski et al. 2008, FEMS Microbiol. Ecol. 66, 567-578;
4) Haenen et al. 2013, J. Nutr. 143, 274-283

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2.

Influences of different feather meal in diets for dogs on praecaecal and total tract digestibility of nutrients – a study using dogs and ileo-caecal fistulated Göttingen mini-pigs

Einfluss unterschiedlicher Federmehle im Mischfutter für Hunde auf die praecaecale- und Gesamtverdaulichkeit der Nährstoffe – eine Studie an Hunden und ileo-caecal fistulierten Göttinger Miniaturschweinen

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Processed poultry byproducts have become an important protein source in the pet food industry, which is forced to enlarge the spectrum of alternative ingredients rich in protein. Unprocessed feathers available around the world are high in crude protein but almost indigestible, which necessitates a hydrolysis process. As the drying conditions seem to play a major role in respect to digestibility, more gently drying techniques were developed during the last years. Substituting a commercial diet for dogs by differently processed feather meal (fm) directly before feeding the apparent digestibility (aD) of nutrients was marginally influenced by the gently processed fm, while the aD dropped markedly after feeding the conventionally processed fm (1). The question of this study was whether the different processing conditions of fm still influence the digestibility of nutrients after the normal extrusion process to which almost every dry food for dogs is subjected to.

Material and Methods: In a cross over model six adult female beagle were fed two extruded compound feeds for dogs, which only differed in the type of added feather meal (fmg= gently processed, two step and short-term drying process at moderate temperatures; fmcon= conventionally processed, long-term drying process at high temperature). XP was set to relatively high levels in order to get clear effects due to the different protein sources. All dogs (8.7 – 12.4 kg) received the same amount of feed. After 5 days of adaption, faeces were collected completely for the following 5 days. In order to determine the praecaecal digestibility (pc DG), the study was carried out additionally on 10 adult mini-pigs (Ellegaard). All animals were fitted with an ileo-caecal fistula. In 5 pigs (PL-pigs) the pancreatic duct was ligated to induce an exocrine pancreatic insufficiency, the other 5 pigs served as control. Both diets were soaked for 10 hours (250g diet + 800 ml water) before offered to pigs. The pc disappearance rate was tested in a screening test model according to (2). Statistical analyses were done using the SAS® software.

Nutrient content (g/kg DM)					Amount of feed (g OS) / day for dogs	Amount of feed (g OS) / meal for pigs
Diet containing	XA	XP	XL	NfE		
fmg (25%)	69.9	373	120	329	146	250
fmcon (25%)	61.0	383	124	364	145	250

Results: In the studies on total tract digestibility in dogs no significant differences between the aD of OM, XP, XL and NfE of both diets were found. Particularly striking are the almost identical results of protein digestibility. Even in the experiments on praecaecal digestibility in mini-pigs no significant differences in the aD of OM, XP and NfE could be found. Again, the digestibility of XP was also very similar in control and PL-pigs, in which in PL-pigs on markedly lower level.

	Total tract digestibility (%)		Praecaecal disappearance rate (%) in mini-pigs			
	Dogs		Control-pigs		PL-pigs	
Paramter	fmg	fmcon	fmg	fmcon	fmg	fmcon
OM	77.3 ± 1.64	76.2 ± 2.70	72.2 ± 1.18	71.8 ± 1.83	45.2 ± 1.61	44.9 ± 2.61
XP	78.1 ± 2.54	78.2 ± 3.91	74.6 ± 1.99	74.4 ± 1.56	24.5 ± 2.20	24.8 ± 5.63
XL	91.3 ± 1.04	90.7 ± 1.50	85.4b ± 5.71	87.5a ± 4.63	29.1a ± 3.85	33.1b ± 2.20
NfE	76,6 ± 1,39	74 8 ± 2,25	69,3 ± 0,85	68,1 ± 2,42	70,9 ± 4,26	69,6 ± 4,22

Different letters indicate significant differences within a group ($p<0.05$)

Conclusion: Although fm_g and fm_{con} differed markedly in their processing conditions, no clear differences between the total tract and pc DG of both diets were found. As the results of (1) could not be reproduced, it must be assumed that further processing conditions must be taken into account when evaluating individual components of compound feeds for dogs. Presumably the extrusion process with associated high temperatures influences the feed materials and thus the nutritive value of feather meal distinctly regardless of the previous processing conditions.

(1) ZEIGER (2016); Proc. Soc. Nutr. Physiol. 25; S. 72 (2) MOESSELER et al. (2015); Gastroenterology research and practice Vol. 2015, ID 871282

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3.

Influence of formula-feeding and *Clostridium difficile*-infection on intestinal microbiota composition and the abundance of antibiotic resistance genes in neonatal piglets

Einfluss einer Formula-Fütterung und Clostridium difficile-Infektion auf die intestinale Mikrobiota und die Häufigkeit von Antibiotika-Resistenzgenen in neugeborenen Ferkeln

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In pigs, spontaneous outbreaks of *Clostridium difficile* infection (CDI) have been increasingly observed in neonatal piglets < 14 days of age. We have recently shown that formula feeding promotes the susceptibility of neonatal piglets to CDI and alters intestinal microbiota composition (1). Although it is clear that antibiotic treatment increases the abundance of antimicrobial resistance (AR) genes in pigs, less is yet known about the resistome development in environment-, diet- or pathogen-induced microbiome alterations. We hypothesized that an imbalanced microbial colonization with CDI might be associated with an expansion of AR genes.

Methods: We analyzed shotgun metagenomes obtained by Illumina NextSeq500 from feces of multiparous lactating sows (S, n=4), pooled feces of their suckling piglets at 5 days of age (SP, n=4), individual fecal samples of the same piglets at 42 days of age (two weeks after the weaning) (WP, n=8), 5-day old isolator-reared and formula-fed siblings (FP, n=3) and FP infected with 10^8 cfu *Clostridium difficile* (FP-CD, n=3). Groups S and WP received commercial wheat-barley-soybean meal based diets, whereas SP exclusively received mothers milk and FP and FP-CD received a formula based on skimmed milk powder and whey (22.6% CP, 20.0% EE, 46.0% lactose per kg DM). The average number of reads per sample was 15 942 818 ± 4 775 046. Quality checked sequences were mapped against reference genomes of 13193 different bacterial and archaeal species taken from NCBI using yara („yet another read aligner“) and further analyzed using the Species Level Identification of Microorganisms from Metagenomes (SLIMM) tool (2). For resistome analysis, we mapped metagenomic reads against the „Comprehensive Antibiotic Resistance Database“ (http://arpcard.mcmaster.ca). Only genes coding for a true antibiotic resistance were used for further analysis, whereas genes related to multidrug resistance, transcription factors or regulating factors were deleted from the initial list. Statistical analyses (group comparisons, cluster analyses, discriminant analyses) were performed in SPSS, SIMCA-P, R and CANOCO.

Results: We identified a total of 1070 bacterial species within 134 genera and 7 major phyla. *Lactobacillus, Prevotella* and *Clostridium* were predominant in S and WP pigs, whereas *Lactobacillus, Bacteroides, Alistipes*, and *Ruminococcus* dominated in SP and in part in FP. A high abundance of *Escherichia, Streptococcus, Shigella, Enterococcus* and *Ruminococcus* was observed in FP-CD and FP piglets. The abundance of AR genes (i.e. *tet*(W), *tet*(Q), *lnu*(C), *lsa*(E), *ant(6)-Ib)* resistance in S, SP and WP metagenomes coincided with the abundance of the bacterial genera such as *Megasphaera, Bifidobacterium, Lactobacillus, Clostridium*, and *Prevotella*, re-establishing these genera as common carriers of these AR genes. In FP and FP-CD piglets, a strong co-occurrence of genes encoding for resistance against aminoglycosides (e.g. *aph(3“)-lb*, *aph(6)-ld*, *ant(2“)-la*), β-lactams ($bla_{\text{CTX-M}}$, bla_{TEM}), fluoroquinolones (*pat*(A) macrolides (*mph*(A)), sulfonamides (*sul1, sul2*), polypeptides (e.g. *pmrB, pmrC, arnA, bac*(A)) and tetracyclines (e.g. *tet*(A-D),) was observed. Similar patterns of co-occurrence have been also recently described in other pig environments (3).

Conclusions: In summary, we could show that formula feeding alone or combined with CDI leads to an immature-type intestinal microbiota and promotes a highly abundant bacterial resistome in neonatal piglets. The relevance for therapeutic resistance or the development of AR gene „supershedders“ later in life needs to be further elucidated.

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This study was supported through EU (Sporebiotic) and DFG (Pi 946/2-1) funds.

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4.

Effect of the gradual replacement of grains with bakery products on ruminal fermentation, nutrient degradation and microbiome *In vitro*

Einfluss des sukzessiven Ersatzes von Getreide durch Backprodukte auf Pansenfermentation, Nährstoffabbau und Mikrobiom In vitro

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In many countries, bakery products (BP) are frequently delivered to stores to ensure freshness. Those not sold within a day are excluded from human consumption. Due to the increased availability of BP as feeds for livestock, a new segment of feed industry based on BP has emerged. Yet, there is a lack of information regarding the effects of inclusion of BP in ruminant diets on ruminal fermentation and microbiome. Therefore, the aim of this study was to evaluate the effect of the gradual replacement of grains with BP on ruminal fermentation, nutrient degradation and microbial community using the rumen-simulation technique (Rusitec).
Methods: All diets consisted of hay and concentrate mixture with a ratio of 42:58 (DM-basis), but differed in the concentrate composition with either 45% cereal grains (wheat and rye) or BP, whereby 15, 30, or 45% of BP were used in place of cereal grains. The BP used was a mixture of leftover materials (mainly bread, cakes, and biscuits) collected from Viennese bakeries and supermarkets and the same charge was used in all approaches. A 12-fermenter Rusitec system was used in 3 randomized 10-day runs (n = 9 per treatment), each run including a five-day adaption- and a five-day sampling-period. In each run 12 g DM of the respective experimental diet was added daily to each of the 3 fermenters per diet. During the sampling period, rumen fluid from each fermenter was collected daily before feeding. Short-chain fatty acids were analyzed by gas chromatography, and microbiological analyses were conducted by 16S rRNA Illumina MiSeq sequencing. An analysis of variance was performed with SAS (9.2) to test the effects of dietary treatment, as well as linear and orthogonal effects of the BP inclusion.
Results: Inclusion of increasing levels of BP in the diet linearly enhanced ruminal degradation of starch from 84% (CON) to 96% (45% BP). The degradation of crude protein and fiber was lowered in diets containing the BP ($P < 0.01$). The pH of CON was 6.63 on average and differed only from 45 % BBP (6.59; $P = 0.02$). The formation of methane was lowered by 14% in the 45% BP diet compared to all other diets ($P < 0.05$). The ammonia concentration was decreased in 30% BP (-22%) and 45% BP (-34%) compared to CON ($P < 0.01$). Also, BP feeding shifted fermentation profile towards propionate at the expense of acetate, causing a decrease in the acetate: propionate ratio from 2.63 (CON) to 1.91 (45%BP, $P < 0.01$). Moreover, isobutyrate linearly decreased with increasing BP inclusion level ($P < 0.01$). According to next-generation sequencing, the microbial diversity decreased in the 45% BP diet compared to CON ($P < 0.05$). The replacement of cereal grains with BP went along with an increased abundance of the genera *Prevotella*, *Roseburia*, and *Megasphaera* while decreasing *Butyrivibrio* and several OTUs belonging to the family of *Ruminococcaceae* ($P \leq 0.10$).
Conclusions: The inclusion of BP up to 30% of the DM had no detrimental effects on pH, fiber degradability, and microbial diversity, and enhanced propionate production. However, a higher replacement level (45%) impaired ruminal fermentation traits and fiber degradation.

This study was funded by the Viennese Chamber of Commerce (Wirtschaftskammerpreis 2016).

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5.

Validation of the RumiWatchSystem for measuring chewing activity in dairy cows

Validierung des RumiWatch Systems zur Messung der Kauaktivität bei Milchkühen

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Measurement of chewing activity provides useful information about cow´s health and behavior. The study was conducted to investigate the suitability of the RumiWatch noseband pressure system (RWS, RumiWatch®, Itin + HochGmbH, Switzerland) for automated chewing behavior classification.

Methods: In an eight-week experimental period, 14 lactating German Holstein cows were housed in a free stall barn and randomly assigned to two groups of 7 cows. Cows of each group were fitted with 7 noseband pressure sensors on a weekly rotation basis. The RWS comprises of a noseband sensor with a data logger and a liquid filled pressure tube with a built-in pressure sensor to register chewing activity. Then, chewing data is classified in parameters such as ruminating, eating, drinking and other activity by the RumiWatch Converter software (RWC, V0.7.4.5). Within the first 3 days of each experimental week, cows were allowed to adapt to the halter. In time-synchronized parallel registration, we recorded the mentioned chewing parameters in time slots of 2 minutes each per cow at 5 hours a day in the following 3 days recording period using scan-sampling direct observation by one observer as the reference method. Finally, sensor data were classified and aggregated in 1 minute summaries by RWC, recapped in units of 2 minutes and plotted against data from direct visual observation to calculate a confusion matrix, Matthews Correlation Coefficient (MCC) and Bland-Altman-Analysis to assess system´s accuracy.

Results: Validation results of the RWS classification data confirmed the highest MCC for ruminating (MCC = 0.86), followed by other activity, eating and drinking (Tab. 1) when compared to direct observation. All parameters attest the RWS and RWC (V0.7.4.5) an accurate classification performance confirming high sensitivities with the exception of drinking, which is not accurately distinguished indicated by a low precision value (Tab. 1). The causal relationship to the misclassification could be explained in similarities of jaw movement patterns between drinking, eating, and other activities. As a result, drinking was especially assigned to eating. The Bland-Altman-Analysis plots the mean of measured classification behavior between the RWS and direct observation per day against their differences around the 95 % confidence interval. A bias deviating from zero suggests a systematic error, e.g. Ruminating is overestimated 5.3 minutes per day by the RWS compared to direct observation, which is, however, relatively small but still significant ($P < 0.001$, Tab. 1).

Table 1: Comparison analysis of classification parameters between RumiWatch sensor data (1-min resolution, RWC V0.7.4.5) and direct observation (pooled sample, n = 14 cows, 75 hours observation time per cow).

	Confusion Matrix [%]					Bland-Altman-Analysis				
Parameter	Sensitivity	Specificity	negative predictive value	Precision	Matthews Correlation Coefficient	bias	Standard deviation	Lower 95 % CI	Upper 95 % CI	P-value of bias vs 0
Ruminating	95.28	92.31	97.50	86.17	0.86	-5.3	5.1	-15.4	4.7	< 0.001
Eating	80.07	92.78	95.19	72.27	0.70	-3.1	6.6	-15.9	9.8	< 0.001
Drinking	14.24	99.85	98.87	55.26	0.28	1.5	1.5	-1.5	4.4	< 0.001
Other activity	85.99	96.55	88.92	95.54	0.83	7.0	7.5	-7.7	21.6	< 0.001

Conclusion: The validation results of chewing activity verify the RWS as an applicable and accurate measurement technique for scientific approaches, which is attested by high correlation coefficients and small systematic errors. Especially, rumination behavior is well-identified and classified, whereas the generic algorithm of the RWC V0.7.4.5 has to be optimized in order to classify the drinking behavior. However, it is to say that influential factors such as RWC-version and the chosen type of time summary of raw data seem to provide remarkably different measuring performance.

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6.

Microbial protein formation of different carbohydrates *In vitro*

Mikrobielle Proteinbildung aus verschiedenen Kohlenhydraten In vitro

*Pfau F., Hummel J. – Göttingen

Introduction: High yielding dairy cows have a particularly high protein demand, which has to be covered largely by microbial protein (MP) formed in the rumen. The amount of MP depends on energy supply to microbes. Since carbohydrates represent the main energy source for ruminal microorganisms, the question of this study was how the type of carbohydrate (e.g. sucrose, starch, cellulose or pectin) influences the amount of MP.

Material and methods: Sucrose, wheat starch, cellulose and citrus pectin was incubated for 24 h in the Hohenheim gas test (HGT) system (3 runs x 2 syringes). *In vitro* gas production (GP) and ammonium content were measured after 8 and 24 h of incubation. To estimate MP, an approach to estimate utilisable crude protein (uCP) based on the ammonium values was used (1). The MP content of each syringe was calculated in g per kg DM and in g per L GP. For the characteristics of the fermentation curves of the four substrates GP was measured after 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation of two additional HGT runs (2 runs x 3 syringes). The time of half-maximal GP ($t_{1/2}$) was estimated using the equation $y = A\{1\text{-}exp[-b(t\text{-}T)\text{-}c(\sqrt{t}\text{-}\sqrt{T})]\}$ (2). Values of MP for $t_{1/2}$ were calculated using the 8 h and 24 h uCP values with the effective uCP approach (1). For statistical analysis a 2-factorial analysis of variance (substrate, incubation time, substrate*incubation time) was used with consecutive comparison of means (Tukey-Kramer).

Results: Starch and cellulose had higher MP [g/kg DM] values than sucrose for $t_{1/2}$, but values did not differ between the polysaccharides. The MP for $t_{1/2}$ related to GP differed between the very fast fermentable substrates sucrose and pectin and the slower fermentable starch and cellulose. In the table fermentation characteristics and microbial protein (MP) formation of different carbohydrates (comparison of LSmeans) are summarised (Means within a line (a-c) and a column (A-C) with different superscripts differ ($p<0.05$)).

Conclusions: Given the considerable differences in fermentation rate, comparison on basis of $t_{1/2}$ was the most meaningful. While the formation of less MP from sucrose was expected, the lower value for pectin contradicts an older hypothesis stating that ß-glycosidic polysaccharides result in higher MP yield; however, it is in line with a more recent publication (3). The results for MP represent net production (difference between formation and degradation of MP), which at least partly explains higher MP values for 8 h as compared to 24 h for the polysaccharides.

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7.

Wheat bran in diets for laying hens: Effect on nutrient digestibility/retention and adaption time

Weizenkleie im Legehennenfutter: Einfluss auf die Nährstoffverdaulichkeit/Retention und die Adaptationszeit

*Wanzenböck E., Schedle K., Kneifel W. – Vienna

Cereals play an important role in human and animal nutrition. Due to an increasing competition between humans and livestock feeding, the supplementation of feed with side-streams of the Agrifood chain is a sustainable approach for ecologically practicable feeding strategies. Wheat bran is a side-product of the milling industry and accumulates up to 150 million tons per year worldwide. It comprises nutritionally valuable compounds (13-18 % protein, 10-14 % starch, 4 % lipids and 7 % minerals). However, due to its high dietary fiber content, the implementation of wheat bran in laying hens diets is restricted. In spite of several drawbacks resulting from diets containing high amounts of wheat bran, several positive effects of dietary fiber have already been observed. Moderate supplementation triggers the production of short-chain fatty acids in the gut, stimulates the growth of bifidobacteria and lactobacilli and does not impair the performance of laying hens. In this respect, this study was carried out to investigate the influence of diets comprising wheat bran as a dietary fiber source on digestibility, retention and adaption time of laying hens.

Methods: In total, 24 Lohman Brown-Classic laying hens (35 weeks old with an initial body weight of 2000 ± 269 g) were subjected to three diets (0 %, 7.5 %, and 15 % wheat bran, respectively). Each hen was accommodated in one metabolism cage, feed intake was recorded daily and excrements were collected from day 4 to day 9 (period 1) and from day 16 to day 21 (period 2). Feces from one hen were pooled per period and subjected to further analyses. Homogenized samples were dried in an oven for five days at 55°C and after analyzing the samples according to the Weender analysis, starch, neutral detergent fiber (NDF), gross energy and mineral (calcium, sodium, zinc, and phosphorus) analysis were performed. Analyses were performed in duplicates. Data were analyzed according to a two-way ANOVA using the MIXED procedure of SAS Enterprise Guide 7.1.

Results: The supplementation of wheat bran did not affect the digestibility of ether extract (EE) and neutral detergent fiber (NDF) as well as the retention of crude ash (XA), calcium, sodium, and phosphorus. In contrary wheat bran impaired the digestibility of dry matter (DM), starch (XS) and the retention of nitrogen (N) and zinc. The factor period did not affect the digestibility of most nutrients. However, the digestibility of NDF was decreased (-21.2 %) in period 2 and also the retention of sodium (-32.1 %) and phosphorus (-62.3 %) was remarkably lower in period 2.

Conclusion: The adaption time had a significant effect on digestibility for specific minerals like Na and P, indicating that an adaption time of 4-9 days is not sufficient. Furthermore, the supplementation of wheat bran decreased digestibility and retention of most nutrients.

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8.

Studies on occurrence of gastro- and enteroliths in the alimentary tract of adult domesticated ostriches

Untersuchungen zum Vorkommen von Gastro- und Enterolithen im Verdauungstrakt von adulten Farmstraußen

*Schuchmann F., Kamphues J., Pfarrer C. – Hanover

Introduction: Under aspects of comparative nutrition and physiology it is noteworthy that the ostriches are able to utilize plants including roughages (for example corn silage, alfalfa hay) without having teeth, without processes like rumination, without the need of finely ground feed materials. But it is well known since decades that this capacity is based on a continuous ingestion of stones with an unique intensity (1). Aim of present studies was to generate data on amounts, sizes and quality of gastro- and enteroliths in farmed ostriches fed common rations (including whole plant corn silage, alfalfa hay and concentrates). Furthermore continuous offer of „stone material" is widely established, thus the ratio of stones to the „normal" digesta was of interest (2). The terms gastroliths and enteroliths are suggested as more precise descriptions of the origin of the stones.

Methods: For this study the whole gastrointestinal tracts (beak to cloaca) from a total of 10 slaughtered adult ostriches (both sexes) were available. These domesticated German farmed ostriches had an age of 16 months and an average body weight of 115 kg at slaughter. Up to one hour the birds had free access to fed and water. The gastrointestinal tracts were laid up on the section table in a sinuous line and divided in the following compartments (proventriculus, gizzard, small intestines, ceca, the proximal and the distal colon). In a first step the total content of the different compartments was quantified by weighing. Then the total contents were separated by sedimentation (stones/sand at the bottom). In the following step the „inorganic mass" was fractionized by sieve analysis. Finally dry matter as well as ash contents of the digesta were determined by common methods (Weender analysis).

Results: The most important findings (Data: mean ± standard deviation) are summarized in the following table.

localisation/ compartment	total weight (organ + content) (g)	organ weight (empty) (g)	mass/gut fill (g)	´normal´digesta (g)	„stones and sand" (g)	stones´ size, (%)	
						> 3mm	< 3mm
proventriculus	2199(±708)	1243(±188)	956(±591)	723(±639)	234(±244)	97.7(±32.2)	2.31(±2.09)
gizzard	4628(±866)	2950(±453)	1678(±452)	648(±496)	1156(±351)	97.7(±25.2)	2.33(±1.86)
small intestine	2646(±169)	1398(±94.9)	1139(±111)	1139(±111)	-(1)	-(1)	-(1)
cecum, short	479(±126)	102(±15.3)	378(±122)	332(±133)	45.8(±71.2)	78.9(±47.0)	21.1(±33.1)
cecum, long	535(±91.3)	112(±16.2)	423(±88.1)	347(±145)	75.5(±93.2)	69.9(±45.7)	30.1(±61.4)
colon, proximal part	4217(±1102)	843(±160)	3374(±1009)	3355(±1016)	18.9(±29.3)	47.1(±40.7)	52.9(±61.6)
colon, distal part	3114(±1038)	844(±142)	2270(±920)	2265(±917)	5.11(±6.80)	36.4(±35.8)	63.6(±65.4)

(1) Up to now, not quantified

Conclusion: Characterizing the ´normal´ gastrointestinal tract fill it has to be underlined that in the cranial part (proventriculus/gizzard) more than the half (52.7 %) of the gut fill wet weight is represented by „stones". Along the gastrointestinal tract this share is reduced more and more, thus the ratio of course to fine stones is reversed (in comparison to the cranial part of the gastrointestinal tract). Astonishing is the fate of the „stones": Due to processes of abrasion the stones are losing their native size and are excreted predominantly as „sand". Thus due to the mixing/milling the roughage is decomposed/ground in a way that is comparable to the function of a „pebble mill".

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„Up to GfE-conference the missing data on „stones and sand" in the small intestine will be available, thus a statistical analysis regarding diminution along the gastrointestinal tract will be performed for all localisations."

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9.

The digestibility of two extruded complete diets for dogs based on either chicken as a conventional protein source or insect meal from *Hermetia illucens*

Verdaulichkeit von extrudierten Alleinfuttermitteln für Hunde mit Huhn als konventioneller Proteinquelle oder Insektenmehl von Hermetia illucens

*Meyer L., Kölln M., Kamphues J. – Hanover

Introduction: The demand for meat will increase by 75 % from 2005/2007 to 2050 (Alexandratos and Bruinsma 2012). Therefore alternative protein sources for both, humans and animals, have to be found. The aim of this study was to compare complete feeds with different protein sources (insect meal vs. chicken meal) offered as extruded dry dog diets regarding the apparent nutrient digestibility and parameters of faecal quality.
Methods: In a crossover model six adult female beagles (age: 1-3 years) were fed two different extruded complete diets. One contained chicken as the main protein source (per kg DM in the complete diet: OM 929 g; CP 282 g; CF 141 g; NfE 481 g), the other one insect meal from Hermetia illucens (per kg DM in the complete diet: OM 926 g; CP 275 g; CF 183 g; NfE 438 g). The insects were reared with feedstuffs for livestock. After an adaption period of 5 days faeces were collected completely for 5 days and the faecal consistency was classified by the following scores: 1 = very hard; 2 = solid, well formed; 3 = soft, still formed; 4 = poor consistence; 5 = watery diarrhea. Furthermore other faecal parameters like dry matter content and mass were also determined.
Results and discussion: The following table presents the main results of the study.

Table 1: Composition of the diets (both provided by Marsa Pet GmbH; Bornheim, Germany), apparent digestibility, faecal parameters

	Complete diet based on chicken	Complete diet based on insect meal
Diet		
Amount of feed/dog [g DM/5 days]*	660	678
Organic matter [g/5 days]	614	627
Crude protein [g/5 days]	186	186
Crude fat [g/5 days]	92.9	124
N free extracts [g/5 days]	318	297
Faecal parameters		
Mass [g DM/5d]	136a ± 12.3	144a ± 6.99
DM content [%]	26.8a ± 2.43	25.8a ± 1.96
Score (consistency)	2.56a ± 0.42	2.34b ± 0.42
aD (whole diet) [%]		
Organic matter	83.4a ± 1.88	82.1a ± 0.87
Crude protein	78.2a ± 3.19	80.5a ± 2.08
Crude fat	95.3a ± 0.69	96.9a ± 0.91
N free extracts	87.1a ± 1.62	83.2b ± 1.18

* calculated by the maintenance requirement of 0.4 MJ ME/kg bodyweight0.75 per day and dog
aD = apparent digestibility a, b indicate significant differences ($p<0.05$) between the groups/ dietary treatment

The digestibility of crude protein tended to be higher ($p=0.057$) for the diet based on insect meal. The crude fat digestibility was at least as good as the digestibility of the diet containing chicken. It is important to keep the lower content of crude fat in the complete diet containing chicken in mind for this parameter. Concerning the organic matter and the N free extracts the digestibility of the complete feed with chicken was slightly higher, in case of N free extracts even significantly ($p=0.0027$). Comparing the faecal parameters it was observed that the diet with chicken led to a little higher faecal DM content, but significantly less favorable scoring for the consistency ($p=0.0019$).
Conclusion: The complete feed containing insect meal as main protein source showed an at least comparable digestibility for crude protein and crude fat compared to the complete feed containing chicken. In addition to the slightly more suitable faecal consistency these are important reasons to consider insect meal as an alternative to diets containing conventional protein sources.

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10.

Experimental and laboratory studies on effects of the grinding intensity of feed grain (wheat/barley/rye) on „extract viscosity“

Laboranalytische Studien zur Bedeutung der Vermahlungsintensität von Futtergetreide (Weizen/Gerste/Roggen) für die „Extraktviskosität“

*Grone R., Kamphues J., Ratert C., Kölln M., von Felde A., Grone R. – Hanover/Bergen

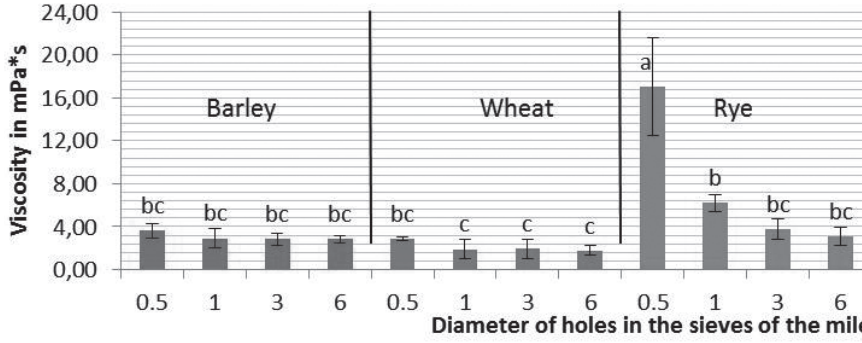
For decades it is known that cereals differ markedly regarding the NSP content [1]. Especially in feeding poultry the extract viscosity became a parameter of interest, (DUSEL et al. 1997)last but not least due the effects within the GIT (passage/digestibility/excreta quality/wet litter). But in pigs also there are interesting side effects, when the grinding intensity is modified. For example the consistency of liquid diets depends on the type of cereals used, the grinding intensity and is altered by soaking, too. The aim of the present study was to compare the extract viscosity when three recent cultivars of barley, wheat and rye were ground by using the identical techniques.

Optimizing feed technology in producing compound feeds has to consider the effects of grinding, enabling high nutrient utilization but also minimizing adverse effects.

Material and Methods: Different barley, wheat and rye varieties were examined (replicates, [KM1] n = 3). For the extract viscosity measurement, the cereals had to be processed first. All three varieties were ground with 1, 3 and 6 mm sieves in the institute‘s own hammer mill. Additionally the samples were crushed by using a Retsch ZM 200 mill (sieve insert 0.5 mm). Subsequently, 5 g of each sample were soaked in 20 ml water and stored at 38 °C for 30 min. After centrifuging the sample at 10000 g for 5 min (Heraeus Biofuge Stratos), extract viscosity was determined by Brookfield Model DV-II + Viscometer. The viscometer was equipped with a spindle (S40) rotating at 10 rpm. After centrifugation the measuring unit was filled with 600 µl of the clear particle-free supernatant.

Statistical evaluation was done by SAS® Ryan-Einot-Gabriel-Welsch multiple Range-Test (SAS®).

[KM2] **Results:** The measured viscosity depended on the type of cereal and the grinding intensity. The rye had a 1.10 – 6.03 times higher viscosity values compared to barley and wheat in each grinding intensity. In addition, it is noticeable that the rye viscosity correlates positive with higher grinding intensity.



Mean ± SD; Superscript letters (a, b, c) show significant differences ($p < 0.05$) between grinding intensities and cereal varieties.[KM3]

Conclusion: At identical grinding there were marked differences between the three grains. Especially it has to be underlined that the extract viscosity differed in rye depending on its grinding intensity. Maybe that some of the adverse effects - known for rye, when it is used in poultry diets – are related to unintended effects on the viscosity. On the other hand some of these effects can be utilized, for example to improve the consistency on liquid diets for pigs; here a more creamy consistency could contribute to avoiding demixing processes, for a longer stay of the digesta in the cranial part of the GIT or could contribute to a higher rate of carbohydrates that enter the hind gut.

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11.

Effect of dietary lignocellulose on the animal performance and intestinal microbiota in slow growing male chickens

Auswirkungen von diätetischer Lignozellulose auf die Leistungsparameter und intestinale Mikrobiota bei langsam wachsenden männlichen Hühnern

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Studies have shown that the dietary supplementation of lignocellulose at low levels did not affect animal performance but can have a direct impact on the intestinal microbiota and the metabolic activity in broilers (1). The aim of this study was to investigate whether the dietary supplementation of lignocellulose at higher inclusion rates influences the animal performance and intestinal microbiota in slow growing chickens.

Methods: A total of fifty nine, ten-weeks-old male chickens (White Leghorn x New Hampshire) were reared in single cages and fed three different diets with varying lignocellulose levels (ARBOCEL®) for a period of 23 days. The diets were based on maize, wheat, soybean meal and soybean oil and were supplemented with either 0.8% (LC 1), 5.0% (LC 2) or 10% (LC 3) of lignocellulose being isonitrogenous and isoenergetic. During the feeding trail various animal performance parameter were recorded. At the end of the trial, chickens were killed and samples of ceacal digesta taken for analyzing ammonia- and lactic acid concentrations (photometric and high-performance liquid chromatographic) as well as short-chain fatty acids (SCFAs) (gas chromatographic). Statistical analyzes were based on Kruskal-Wallis-Test, LC 1: n = 20, LC 2: n = 20, LC 3: n = 19

Results: Dietary lignocellulose had no impact on the chicken‘s bodyweight ($P > 0.700$), weight gain ($P > 0.484$), feed intake ($P > 0.316$) and feed efficiency ($P > 0.298$). The relative proportion of SCFAs, lactic acid and the total amount of fatty acids in caecal digesta of chickens fed LC3 were lower compared to birds fed with LC1 and LC2. In contrast to the other bacterial metabolites, the concentration of acetic acid was higher in birds fed LC3 compared to chicken receiving LC1 and LC2.

Caecal Digesta	LC 1	LC 2	LC 3	SEM	P-value
Total fatty acids, mmol/l	56.8[a]	58.2[a]	44.9[b]	1.44	< 0.001
Relative proportion, mol %					
Acetic acid	66.8[a]	67.1[a]	75.5[b]	0.67	< 0.001
Propionic acid	16.0[a]	16.8[a]	10.9[b]	0.52	< 0.001
i-butyric acid	0.76	0.69	0.59	0.04	0.451
n-butyric acid	14.9[a]	13.8[a]	11.4[b]	0.32	< 0.001
i-valeric acid	0.52	0.50	0.38	0.04	0.318
n-valeric acid	1.07	1.08	1.32	0.06	0.465
Ammonium and lactate, mmol/l					
NH4+	9.46[a]	8.72[a]	7.71[b]	0.44	0.009
L-lactate	1.70[a]	1.34[a]	0.67[b]	0.13	0.010
D-lactate	0.97	1.06	0.42	0.14	0.060
L/D-lactate ratio	8.01	1.95	2.43	1.73	0.290
[a,b] Values with different indexes in a row differ significantly ($P \leq 0.05$)					

Conclusion: The results of this study showed that the dietary inclusion of lignocellulose had no negative impact on animal performance parameters but influenced the ceacal microbial metabolites in chickens. A 10% lignocellulose supplementation decreased the overall amount of SCFAs implying changes in the microbial composition respectively metabolism. In contrast, studies on broilers showed that the dietary inclusion of 0.5 % lignocellulose increased the total amount of ceacal SCFAs, lactic acid and beneficial bacteria may suggest a prebiotic effect of dietary lignocellulose (1). Further quantitative and qualitative analyzes of the ceacal bacteria are needed, in order to clarify whether the dietary inclusion of 10% lignocellulose also led to changes of the microbial composition in chickens.

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12.

Effect of low feed intake levels on digesta passage and nutrient digestibility in Boran steers

Auswirkung limitierter Futteraufnahme von Boranrindern auf Digestapassage und Nährstoffverdaulichkeit

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Cattle in sub-Saharan Africa are regularly exposed to undernutrition, due to limited availability of feed, a low crude protein (CP) and high neutral detergent fibre (NDF) concentration in the dry season. We hypothesised that undernutrition prolongs digesta passage and improves feed and nutrient digestibility. The present study aimed to verify this postulate.

Methods: The trial took place at ILRI Nairobi, Kenya, from 06/2016 to 01/2017. During 4 subsequent periods, 12 Boran steers (183 ±15.2 kg) were subjected to 4 treatments. Per period, 3 animals each were fed at 100, 80, 60 and 40% of their maintenance requirement for metabolizable energy (MER). Each period comprised 21 d adaptation, 7 d sampling of faeces and 14 d recovery. All diets consisted of Rhodes grass hay (3% CP, 77% NDF, on DM basis) - only at 100MER 20% of MER were supplied by a cotton seed meal/ molasses mixture (0.52:0.48). At 8 a.m. of sampling day 1, steers were fed with ytterbium-marked fibre (560 mg/kg live weight, LW) and drenched with cobalt-EDTA (23.6 mg/kg LW). Yb- and Co-containing faeces were collected quantitatively at any moment of excretion for 7 d and analysed [1]. Time of first marker appearance (TT), ruminal passage rate (λ), rumen retention time (CMRT) and total tract retention time (TMRT) of particles (Yb) and liquid (Co) were determined with SAS 9.1 NLIN procedure [2]. PROC MIXED was used to conduct ANOVA with treatment and period as fixed and animal as random effect. Spearman correlation was performed using CORR procedure.

Results: Decreased feed and energy intake resulted in a decelerated passage rate and increased retention time ($p<0.01$), whereby liquid passage parameters were more strongly affected ($r^2 >0.56$) than those of particles ($r^2 >0.48$). With prolonged digesta passage, NDF digestibility first improved but with severe undernutrition decreased ($p<0.001$); likewise, DM digestibility was lowest at 40MER ($p>0.05$). However, correlations between digesta passage parameters and digestibility values were insignificant.

Table 1 Digestibility of diet constituents and parameters of liquid and particle passage through the gastrointestinal tract of 12 Boran steers at four supply levels of their metabolizable energy requirements (MER).

Variable		MER				SEM
		100	80	60	40	
DM digestibility (g kg^{-1})		568	569	560	541	4.71
NDF digestibility (g kg^{-1})		563^{a}	608^{b}	597^{b}	581^{ab}	5.26
Liquid digesta passage	λ_l (% h^{-1})	10.5^{b}	8.8^{a}	8.2^{a}	7.8^{a}	0.32
	TT_l (h)	7.0^{a}	7.9^{ab}	9.1^{b}	11.0^{c}	0.34
	$CMRT_l$ (h)	19.1^{a}	23.3^{b}	25.6^{b}	26.2^{b}	0.77
	$TMRT_l$ (h)	26.1^{a}	31.2^{b}	34.7^{bc}	37.2^{c}	0.88
Solid digesta passage	λ_s (% h^{-1})	3.5^{b}	2.8^{a}	2.8^{a}	2.6^{a}	0.09
	TT_s (h)	14.3^{a}	15.5^{ab}	17.5^{b}	20.8^{c}	0.60
	$CMRT_s$ (h)	59.4^{a}	72.1^{b}	73.6^{b}	78.9^{b}	2.18
	$TMRT_s$ (h)	73.6^{a}	87.6^{b}	91.1^{b}	99.6^{b}	2.42

Least square means, standard errors of means (SEM).Within rows, means with different superscripts differ at $p<0.05$.

Conclusions: Even though declining feed and energy intake decelerated gastrointestinal digesta passage, DM and NDF digestibility were reduced at the lowest supply level. This might be due to the combined effect of severe energy deficiency and low dietary CP content leading to suboptimal rumen conditions.

Acknowledgements: This study was funded by BMZ (Grant No. 5521914).

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13.

Effect of rearing environment and diet on microbial colonization patterns in neonatal piglets

Einfluss von Umwelt und Diät auf die mikrobiellen Besiedlungsmuster in neugeborenen Ferkeln

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The early-life environment and diet are main factors driving the *de novo* establishment of the neonatal intestinal microbial ecosystem. We hypothesized that both, artificial rearing and formula feeding in neonatal piglets - an increasing trend in swine production due to hyperprolific sows - would have strong impact on microbial colonization patterns during the first two weeks of life.

Methods: A total of n=48 newborn piglets were randomly allocated into four different groups (n=12 each) in a 2 x 2 arrangement with environment (sow-reared (Sow) or artificial rearing unit/isolator (Iso)) and diet (sow milk (SM) or formula (FO)) as main factors. Formula was based on skimmed milk powder and whey. Sow milk for feeding of piglets kept in artificial rearing units (group Iso-SM) was obtained in the previous lactation from the same mothers. Piglets in Sow-FO groups were removed twice daily from their mother and fed formula, but effectively received both, sow milk and formula. On days 1, 2, 3, 5, 7, 10 and 14 of life, faecal samples were collected for further analysis. Total DNA was extracted and used for amplification of the V3-V4 region of the 16 rRNA gene followed by Illumina sequencing of amplicons. Obtained sequences (n= 44,830±10,919 per sample) were quality checked and analysed using the MG-RAST server (http://metagenomics.anl.gov/). Diversity measures and changes of genus, family and OTU level abundances were evaluated using generalized linear models with environment and diet as main factors in SPSS. Identification of unique bacterial taxa among groups was performed by Partial Least Squares-Discriminant Analysis (PLS-DA) with variable importance in projection (VIP) scoring using SIMCA-P.

Results: Starting with an average richness of n=126±24 species in faecal samples of all groups obtained on day 1, bacterial richness in piglets kept in isolators did not change significantly over time (n=151±24 and 116±24 for Iso-SM and Iso-FO, respectively) as compared to piglets reared with their mothers, showing increasing richness until 14 days of age (n=219±49 and 201±41 for Sow-SM and Sow-FO, respectively). At phylum level, significant effects of environment (Fusobacteria, Bacteroidetes) and diet (Proteobacteria) were observed, whereas significant effects of environment (e.g. *Fusobacterium, Prevotella, Paralactobacillus, Collinsella*) and diet (e.g. *Lactobacillus, Clostridium, Bacteroides, Bifidobacterium*) were observed at genus level. Dynamic changes of taxa abundance were observed over time. These dynamic changes were more obvious in sow-reared piglets as compared to isolator-reared piglets, where initially present taxa persisted or even increased in abundance over time. As an example, an early-life gut colonizing species, *Fusobacterium necrophorum*, was highly abundant in all animals at day 1, disappeared in sow-reared piglets but increased in abundance in isolator-reared piglets. As expected, the majority of unique taxa were found in sow-reared piglets. However, some bacteria were solely associated with isolator-rearing including *Lactobacillus spp., Enterococcus spp., Shigella spp*., and *Campylobacter spp*. Finally, PLS-DA and VIP scoring identified group-specific differences in taxa abundance with a major influence of diet. This included mainly members of the genera *Bacteroides, Prevotella, Clostridium, Lactobacillus, Streptococcus, Veillonella* and certain proteobacteria.

Conclusions: Both, the environment as well as the diet affect early-life microbial colonization patterns. Delayed gut colonization and limited bacterial dynamics are likely due to limited microbiota exposure in isolator-reared piglets, and some effects were further amplified by diet.

This study was supported by the German Research Foundation (Pi 946/2-1).

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14.

Effect of nitrogen supply, nitrogen source and buffer medium on *In vitro* rumen gas production and *In vitro* degradability of different carbohydrates

Einfluss von Stickstoffmenge, -quelle und Puffermedium auf die ruminale Gasproduktion und Abbaubarkeit verschiedener Kohlenhydrate In vitro

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Rumen nitrogen balance (RNB) is an indicator for the ratio of nitrogen (N) to energy supply available to symbiotic rumen microorganisms. A surplus in N supply leads to wasteful N emissions which are of ecological concern. A reduction in N supply might increase efficiency of N use but might impair the fermentation of carbohydrates (CHO). In addition, the sources of N and energy (i.e., CHO) affect the rate of rumen fermentation. The aim of this study was to investigate how a negative RNB affects rumen degradation of different CHO and N sources *In vitro*. Two commonly used buffer media were chosen to test, if the N content of the buffer medium impacts on the results of the *In vitro* incubation.

Methods: Various substrate mixtures were incubated using the Hohenheim gas test (1) with 10 ml of rumen fluid and 20 ml of either Menke & Steingass buffer (MS; 0.71 g N/l) or of McDougalls buffer (MCD; 0 g N/l) (2). Each substrate mixture consisted of 100 mg of grass hay and 100 mg of either one of the following three combinations of N and CHO sources: wheat gluten-maize starch (WG-MS), urea-maize starch (U-MS), urea-cellulose (U-CEL), at two RNB levels: balanced (0 g/kg dry matter (DM)) or negative (-9 g/kg DM) where actual RNB was determined after incubation (3). Incubations were run in triplicate on two different days. Gas production and concentrations of short-chain fatty acids were determined after 24 h of incubation. True substrate degradability (4) was calculated after digesting the fermentation residues in an ANKOM fibre analyser with neutral detergent solution. The main effect of buffer medium, CHO or N source, RNB level, and interactions thereof were statistically tested using SAS V9.4.

Results and Discussion: Buffer medium significantly affected gas production and true substrate degradability. Gas production was higher with MS buffer ($P<0.01$) than with MCD, indicating that fermentation was N limited. This is supported by the observation, that true substrate degradability of U-MS and U-CEL in MCD was higher ($P<0.01$) with balanced RNB than with negative RNB. No significant difference in true substrate degradability between RNB was observed using MS buffer indicating that the N content of MS changed the RNB towards the positive scale providing sufficient N to rumen microorganisms. Gas production was higher at a negative RNB with WG-MS and U-MS, but not with U-CEL diets irrespective of the buffer used ($P<0.01$), likely due to a higher proportion of degradable CHO. Branched chain fatty acids (BCFA) proportions, indicators of amino acid degradation, were higher at a negative RNB with WG-MS as opposed to U-MS diets ($P<0.01$) as the former being true-protein and latter being a non-protein N source. Using MCD, true substrate degradability was lower but gas production was higher on a negative RNB and as a consequence the partitioning factor (truly degraded substrate/gas production) was significantly affected by the RNB.

Conclusions: In summary, true degradability of substrate mixtures and partitioning of nutrients were affected by RNB suggesting that reducing the N supply affects CHO fermentation and/or microbial protein synthesis. As the choice of the buffer significantly affected the results, the N content of the buffer medium has to be considered in RNB calculations in *In vitro* incubations.

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15.

Influence of lowering the level of specific amino acids in the diet on the course of an experimental *Campylobacter jejuni* infection in broilers

Einfluss einer Reduktion spezifischer Aminosäuren im Alleinfutter von Broilern auf den Verlauf einer experimentellen Infektion mit Campylobacter jejuni

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Campylobacteriosis is the most frequently occurring bacterial zoonosis in the European Union. Infections are often linked to the consumption and handling of poultry meat [1]. The metabolism of *C. jejuni* relies on the utilisation of amino acids, mainly on serine, aspartate, glutamate and proline, which are preferred in this order [2]. These amino acids are also most frequently present in both, the mucus layer and the excreta of poultry. The hypothesis of the study was that *C. jejuni* prevalence and excretion can be reduced by lower levels of specific amino acids in the diet leading to lower availability of amino acids from the digesta and mucus for the pathogen.

Methods: After the fourteen-day rearing phase, 300 ROSS 308 broilers of both sexes were randomly subdivided into 20 subgroups in a 2x2 factorial design with two different diets and a different infections modus (SPCN-Standard Protein, Campylobacter Negative; LPCN-Low Protein, Campylobacter Negative; SPCP-Standard Protein, Campylobacter Positive; LPCP-Low Protein, Campylobacter Positive). The standard diet (SP-diet) was designed in accordance with a commercially available standard fattening diet (per kg DM: 14.3 MJ ME, 212 g XP, 81.0 g XL, 10.2 Ca, 7.68 P, 1.85 g Na, 8.66 g K). The low-protein diet (LP-diet) contained a smaller amount of soybean meal and therefore had a reduced crude protein content (per kg DM: 14.3 MJ ME, 190 g XP, 71.7 g XL, 12.1 Ca, 6.59 P, 1.96 g Na, 8.34 g K). The growth limiting amino acids lysine, methionine, threonine, isoleucine, valine and arginine were added to the LP-diet in higher proportions than to the SP-diet to meet identical concentrations. Concentration of serine, aspartate, glutamate, proline and other non essential amino acids was reduced. All diets were offered ad libitum.

The animals were adapted to the feed for seven days before three animals (seeder) of every group were experimentally infected with *Campylobacter jejuni* (4.17±0.09 log 10 CFU/animal). On day 1, 2, 3, 4, 7, 14 and 21 after experimental infection cloacal swabs were taken from all animals, excreta samples of seeder animals were analysed quantitatively. Total mucin was determined in pool samples of excreta separately taken at d 20, d 28 (only SPCN and LPCN), d 35 (only SPCN and LPCN) and d 42. The microbiological tests were applied in accordance to DIN EN ISO 10272-1. Goblet cells of 5 caecal crypts each were counted. Subsequently, an average crypt depth of about 250 µm was calculated from the crypt depths. This mean crypt depth was defined as the standard crypt depth. The number of goblet cells of the individual, measured crypts of each sample was converted to this depth for better comparability. Statistics were done by 1-way-ANOVA and Pearson‘s chi-squared test ($p < 0.05$).

Results: The final body weights surmounted breeder“s perspectives. Diet had no influence on the development of the body weight in artificially *C. jejuni* infected animals, where in the not artificially infected animals, the use of the LP-diet led to lower final body weight (d 42, in g; SPCN: 3256±366a, LPCN: 3088±370b, SPCP: 3164±385ab,LPCP: 3124±469ab). Due to the reduced CP-intake a significant decrease ($P<0.05$) in average crude mucin in excreta (55.7 ±8.23 g/kg DM and 51.9 ±7.62 g/kg DM, respectively) and a significant decrease ($P<0.05$) in goblet cell number in crypts of caeca (15.1 ±5.71 vs. 13.6 ±5.91 goblet cells/crypt) were observed. In groups receiving the test diet, the excretion of *C. jejuni* was significantly reduced in seeders by 1.9 log10 CFU/g excreta at day 23 (3.38a±2.55 versus 1.47b±2.20; $P=0.0333$) and the spread of infection was also delayed. At day 25, prevalence of *C. jejuni* in cloacal swabs amounted to 53.3 % in the group fed the test diet and 75.7 % in the control group, respectively ($P<0.05$).

Conclusion: The prevalence of Campylobacter could be reduced through the experimental diet in the initial phase of infection. In summary, a modulated amino acid pattern in the broiler diets had an effect on a *C. jejuni* infection. This optimisation could contribute to a development of an effective feeding strategy to reduce the prevalence of *C. jejuni* infection in chickens.

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16.

Protease and phytase supplementation effects on microbiota composition in the ileum and amino acid digestibility in broiler chickens

Wirkungen von Proteasen und Phytase auf die Zusammensetzung des Mikrobioms im Ileum und die Aminosäurenverdaulichkeit bei Broilern

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Feed enzymes such as proteases and phytases are claimed to increase overall efficiency of protein utilisation in broiler chickens through increased digestibility of amino acids (AA). The intestinal microbiota contributes to digestion because it converts ingested substrates into short chain fatty acids, amines, AA and other compounds that affect the host [1]. However, enzyme supplements may modify enzyme and proteolytic activity which can affect microbiota composition. Thus, the aims of this study were to determine how enzyme supplements affect the microbiota composition in the ileum of broilers and whether effects are related to differences in precaecal (pc) AA digestibility.

Methods: Unsexed Ross 308 hatchlings were placed in 72 pens in groups of 15. A commercial starter diet was provided until experimental diets were used starting on day 14. The 8 nutrient-adequate experimental diets were mainly based on maize and soybean meal. Treatments involved the control diet without enzyme supplementation, protease A (Meiji) at 25 and 200 mg/kg, protease B (Cibenza) at 500 and 4000 mg/kg, protease C (Ronozyme ProAct) at 200 and 1600 mg/kg and phytase (Natuphos E) at 1500 FTU/kg. Diets were provided in mash form for ad libitum consumption and allocated to 9 pens each. Birds were asphyxiated on day 21, the content from the terminal ileum obtained, pooled on a pen basis and stored at -80°C. Determination of pc AA digestibility followed standard procedures. Total nucleic acids were obtained with a commercial kit and 16S rRNA gene Illumina amplicon sequencing was used to characterize microbial assemblages [2]. Multivariate statistical analysis was performed with microbiota data [2].

Results: When compared to the control, a significant increase in growth and feed efficiency of broilers was caused only by phytase and the high dosage of protease C. Average pc digestibility of essential AA was 82% (control), 84% (high dosage of protease C), and 85% (phytase). Differences from the control diet were significant for most of the AA in these two treatments. Other treatments did not cause a consistent effect on pc AA digestibility. A total of 1021 operational taxonomic units were identified in the whole dataset. Firmicutes was the most abundant phylum across all diets (> 98%). A significant difference of the bacterial profiles at genera level was observed among the 8 treatments (p=0.024). *Streptococcus* was contributing to this dissimilarity showing higher abundance with low and high dosage of protease B (24% and 30%), when compared to 13% in protease C at high and low dosages. *Lactobacillus* was showing the highest abundance across all diets and revealed to be negatively correlated with other genera (p<0.05). In protease C, *Lactobacillus* accounted for 77% and 64% of the total community at low and high dosages respectively, while it was detected with 38% abundance upon phytase addition, and 43% and 56% upon protease B addition at the two dosages. Uncultured *Clostridiaceae* was more abundant with protease B at low (20%) and high dose (10%) and phytase (15%) when compared to protease C, where it was only detected in lower abundance (1-4%). Unclassified Clostridiales Incertae Sedis XI was detected in higher abundance upon phytase addition (13%), followed by 6% abundance in protease C at high level and between 0.4% and 3% in the other diets.

Conclusions: Supplements of proteases and phytase affect main bacterial groups in the ileum of broilers. Enzyme effects on the microbiota seemed not to be related to pc amino acid digestibility. Effects of protease supplementation on pc AA digestibility are product specific and dose dependent.

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17.

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

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

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Neonatal piglet survival has decreased during the last 20 years with only 44% of low birth weight (LBW) piglets (< 1 kg) surviving until weaning (1), contributing to the high pre-weaning piglet mortality of up to 35% (2). To date, data on plasma metabolic profiles during the early postnatal period in LBW and normal birth weight (NBW) piglets are scarce. The objective was to investigate the effect of birth weight on growth parameters, plasma metabolites and free amino acids (AA) concentrations in the early postnatal period. We hypothesize that low birth weight changes plasma metabolic and AA profiles.

Methods: At birth, 12 pairs of male German Landrace litter mates born to first parity sows were selected and suckled by their dams for 5 days, in standardized litters of 12 piglets/sow. Each pair had one LBW (1.08 kg ± 0.12, n=12) and one NBW (1.49 kg ± 0.13, n=12) piglet. Piglet body weight was measured daily. Crown-rump length, abdominal circumference and rectal temperature were measured at birth (day 0) and 5 days postnatal (dpn), when piglets were killed. Plasma was collected 4 h after birth via venipuncture, and at 5 dpn via cardiac puncture, to analyse plasma metabolites and free AA concentrations. Data was analysed using the MIXED procedure of SAS, and where applicable, with repeated measures. Least square means were separated using the Tukeys test (P<0.05).

Results: From birth until 5 dpn, LBW piglets were lighter (P<0.01) and had reduced (P<0.01) daily body mass gain (g/day) compared with NBW piglets. At birth and 5 dpn crown-rump length (P<0.01; birth = 10.1%, 5 dpn = 11.4%), abdominal circumference (P<0.01; birth = 13.6%, 5 dpn = 10.5%) and body mass index (kg/m2: birth, P=0.02; 11.7%, 5 dpn, P=0.08; 7%) were smaller compared with NBW piglets. No difference in ponderal index (kg/m3) or rectal temperature was observed between LBW and NBW piglets (P>0.10). At 4 h after birth, concentration of plasma inositol was higher (4.7 vs 3.0 µmol/L; P<0.01) and non-esterified fatty acids (NEFA, 161 vs 206 µmol/L; P=0.05) and total protein were lower (27 vs 32 g/L; P<0.01) in LBW piglets. At 5 dpn, plasma triglyceride levels were higher in LBW compared with NBW piglets (1.1 vs 0.8 mmol/L; P=0.04). No difference in the plasma concentrations of fructose, glucose, lactate, urea, albumin or cholesterol was observed between LBW and NBW piglets, at both sampling time points. At 4 h after birth, plasma concentrations of ornithine (80 vs 102 µmol/L; P=0.04), cysteine (54 vs 66 µmol/L; P=0.05) and glutamine (741 vs 907 µmol/L; P=0.09) were lower in LBW piglets compared with NBW piglets. At 5 dpn, the plasma concentrations of glycine (629 vs 839 µmol/L; P=0.02), 3-methyl-histidine (7 vs 10 µmol/L; P<0.01), hydroxyproline (247 vs 310 µmol/L; P=0.01), and taurine (103 vs 136 µmol/L; P=0.02) were lower and glutamate (231 vs 202 µmol/L; P=0.09) was higher in LBW compared with NBW piglets.

Conclusions: The observed plasma metabolic differences are indicative of reduced colostrum intake and altered glucose and lipid metabolism in LBW piglets. At 4 h after birth, lower concentrations of total protein, ornithine, and cysteine indicate reduced colostrum intake, whilst lower glutamine and NEFA levels might point to a lower endogenous glutamine synthesis, and a lower body fat content in LBW piglets, respectively. In contrast, higher plasma inositol suggests altered glucose metabolism (3). Changes in plasma AA concentrations at 5 dpn may suggest that LBW piglets had lower muscle catabolism and bile acid conjugation. The elevated levels of triglycerides might perhaps reflect a higher hepatic lipid infiltration in LBW piglets.

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18.

First investigations on the optimal methionine:cysteine ratio in diets for growing chickens with a *Tenebrio molitor* press cake as an alternative protein source

Erste Ergebnisse zum optimalen Methionin:Cystein Verhältnis in Futtermischungen für Masthähnchen mit Tenebrio molitor Presskuchen als alternative Proteinquelle

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One of the potential alternative protein sources for the substitution of soybean meal (SBM) is the yellow mealworm *Tenebrio molitor* (TM). The current study aimed to evaluate the most efficient dietary Met:Cys ratio in diets with complete replacement of SBM by a press cake from TM.

Methods: 240 male day-old broiler chickens (Ross 308) were randomly allotted to six diets (48 pens; 5 birds per pen). The growth study was divided into a starter (day 1-21) and a grower period (day 22-35). Diets A-E contained 16.00 / 14.88 % (starter/grower) TM press cake (70.66 %CP, 10.36 %EE in DM) and differed only in their Met:Cys ratio (from 40:60 to 60:40, see table). SBM was the reference protein source in diet F (Met:Cys ratio 50:50). The sulfur containing amino acids (SAA) ratio to lysine was fixed at 0.50 in order to ensure the limiting position of SAA in each of the diets for further model applications. Zoo-technical parameters (growth, feed intake, FCR, mortality) were recorded weekly. After slaughtering (day 36), pooled ileal samples (n=5) were collected from the last ⅔ between Meckel´s diverticulum and 2 cm before the ileo-caecal junction, but only for diets C (TM 50:50) and F (SBM 50:50), respectively. Statistical analysis was conducted by SPSS.

Results: Results of the whole growth period (day 1-35) demonstrate (table) that diet C (TM 50:50) yielded superior growth data, but not significantly different from diets D (TM 55:45), E (TM 60:40) and F (SBM 50:50). Diet A (TM 40:60) provided the lowest growth response. The highest feed intake was observed in diet F (SBM 50:50), but not significantly different from diet C (TM 50:50). Accordingly, diet C (TM 50:50) improved FCR data significantly as compared to the SBM diet F, but means were only numerically different from diets B (TM 45:55), D (TM 55:45) and E (TM 60:40).

Diet	A	B	C	D	E	F
Met:Cys ratio	40:60	45:55	50:50	55:45	60:40	50:50
Final BW (g)	1379^{a} ± 204	1830^{b} ± 177	2378^{c} ± 221	2174^{c} ± 122	2185^{c} ± 165	2247^{c} ± 119
BWG (g/d)	38^{a} ± 6	51^{b} ± 5	67^{c} ± 6	61^{c} ± 4	61^{c} ± 5	63^{c} ± 3
Feed intake (gDM/d)	59.8^{a} ± 10.5	78.1^{b} ± 4.5	89.0^{bc} ± 11.8	83.7^{b} ± 4.5	83.7^{b} ± 6.3	100.7^{c} ± 9.6
FCR (g/g)	1.58^{ab} ± 0.19	1.55^{abc} ± 0.17	1.34^{c} ± 0.15	1.38^{bc} ± 0.04	1.37^{bc} ± 0.06	1.61^{a} ± 0.17

Means (± SD), different superscript letters reveal significant differences between diets ($p<0.05$).

The results of apparent precaecal digestibility (apcD) are summarized in the following table (means ± SD) and indicate no significant difference between both of the diets under study.

apcD (%)	CP	Met	Cys	SAA	Lys
Diet C (TM 50:50)	74.6 ± 2.2	75.8 ± 4.6	68.5 ± 4.4	72.2 ± 4.5	79.2 ± 3.5
Diet F (SBM 50:50)	75.2 ± 1.2	80.6 ± 1.1	60.9 ± 2.0	70.7 ± 1.6	80.8 ± 1.2

Conclusions: Superior zoo-technical data were observed with the TM diet and a Met:Cys ratio of 50:50. However, a higher Met ratio up to 60% of the SAA did not significantly impair growth data. This observation needs to be discussed in context with earlier results. The complete substitution of SBM by a press cake of TM provided no significant effect on apparent precaecal digestibility of CP and individual amino acids under study. The insect meal under investigation is a very promising alternative protein source as compared to SBM as reference.

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19.

L-Valine requirements of broilers in starter period, 0-12 days

L-Valin Bedarf des Masthähnchen in der Starter Phase (0-12 Tage)

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Background: L-Valine is likely the fourth limiting amino acid in Corn soybean meal (CSBM) based diets in broilers (1). Among all three branched chain amino acids (BCAAs), the L-Leucine is abundantly available in raw materials, which contributes in the estimation of the requirement of L-Isoleucine and L-Valine, as they share the same metabolic pathways (to a certain extant) in animal body (2). Identification of ideal ratio among BCAAs themselves and to the Lysine has always been complex. The objective of the present experiment was to determine the optimal dVal: dLys ratio in meat type chickens through the starter period (0-12 d).

Methods: A total of 960 male Cobb 500 broilers were housed in 48 floor pens (20 birds per pen). The basal diet was comprised of corn, wheat, SBM and peas. Out of eight dietary treatments (including each positive control and basal diet) six test diets were created by gradual supplementation of L-Valine to the basal diet. The basal diet with 19.0 % CP and 11.16 g/kg of dLys contained a dVal: dLys ratio of 0.63 and the test diets contained a ratio of 0.68, 0.73, 0.78, 0.83, 0.88 and 0.93 (calculated values). A positive control diet (19.0 % CP) was formulated to contain a dVal: dLys ratio of 0.80. The other essential amino acids were formulated to be around 5% above the required values. Tabular values of the digestibility coefficients were used, developed through internal experiments at Schothorst Feed Research. In order to avoid negative interactions between BCAAs, maximum dLeu: dLys and dIle: dLys ratios were considered as 1.09 and 0.67 in the basal diet respectively. The raw data was analysed through ANOVA, whereas, different regression methods were applied to identify the optimal dVal: dLys.

Table 1: Statistical means of the performance parameters (0-12 d)

Treatment	Total L-Val (Analyzed=g/kg)	Val:Lys (Analyzed)	FI (g)	BW (d 12) (g)	FCR
1 (BD)	9.10	0.69	418	383^{a}	1.214^{e}
2	9.70	0.73	426	399^{c}	1.183^{bc}
3	10.30	0.78	429	398^{bcd}	1.199^{cde}
4	11.20	0.84	430	406^{d}	1.173^{ab}
5	11.10	0.84	422	401^{cd}	1.164^{a}
6	11.90	0.90	427	393^{bc}	1.207^{de}
7	12.60	0.95	417	389^{ab}	1.193^{cd}
8 (PC)	11.40	0.81	430	403^{cd}	1.183^{bc}
LSD			12.16	9.794	0.0183
P-value			0.174	< 0.001	< 0.001

$^{a-e}$ Figures with different superscripts are statistically significant (P<0.05)

Results: The birds demonstrated a significant (P<0.05) response to the supplementation of L-Valine to the basal diet (Table 1). Based on the ANOVA the maximum response to the body weight (406 g) and FCR (1.16) was achieved at 11.20 g and 11.10 g Val/kg of feed in the present study. Whereas, when the data was evaluated through the regression analysis, the optimal dose-response relationships between graded dVal: dLys ratios for BWG and FCR was 0.78:1.00 and 0.80:1.00 respectively.

Conclusion: The maximum response was about 5.66% better than the BD in starter period, however more evident response is expected in the grower and finisher periods. The average of the requirement for maximum body weight gain and FCR lies at the dVal: dLys ratio of 0.80:1.00 in the period 0 to 12 days in broilers. However, additional data for entire growing period is required to suggest more elaborate requirements of L-Val in fast growing chickens.

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20.

Effect of low crude protein concentrations and varying glycine and serine concentrations on growth and nitrogen efficiency in broilers

Einfluss von niedrigen Rohproteinkonzentrationen und unterschiedlichen Glycin- und Serinkonzentrationen auf das Wachstum und die Stickstoffeffizienz von Broilern

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Glycine (Gly) and serine, commonly considered together as Gly equivalents (Glyequi), are the first-limiting nonessential amino acids (AA) when the crude protein (CP) concentration of broiler diets is reduced [1]. However, it is not known to which level the CP concentration can be reduced without impairing growth, and which Glyequi concentration is needed at this level. This study evaluated low CP and varying Glyequi concentrations in broilers.

Methods: Male Ross 308 hatchlings were raised in floor pens for 7 days and received a commercial starter diet. On day 7 of the experiment, 10 broilers were randomly allocated to 84 cages each and received one of 12 dietary treatments in 7 replicates until the end of the experiment on day 21. Three corn-soybean meal-based diets with 163 (CP163), 147 (CP147), and 132 (CP132) g/kg of CP were formulated. Free Gly was added to each diet to achieve Glyequi concentrations of 11.9, 14.9, 17.9, and 20.9 g/kg. Concentrations of essential AA were maintained constant by using free AA in variable proportions. Concentrations of lysine and methionine+cysteine were 10.9 and 7.9 g/kg, respectively. Birds and feed were weighed on day 7 and 21. Excreta were collected quantitatively in 12 h intervals from day 18 to 21. Results were statistically analysed by two-way ANOVA using SAS 9.3.

Results: Significant interaction effects were observed in all traits. Reduction of CP decreased average daily gain (ADG), average daily feed intake (ADFI) and gain:feed ratio (G:F). Addition of Glyequi increased ADG and G:F in CP132. The G:F in CP147 and CP163, and ADG in CP147 were increased up to a Glyequi concentration of 14.9 g/kg. Addition of Glyequi had no effect on ADG in CP163. Additional Glyequi influenced ADFI in CP132, but not in CP147 and CP163. Efficiency of N utilisation was highest at Glyequi concentrations of 11.9 g/kg and 20.9 g/kg in CP132. In CP147 and CP163, N efficiency decreased in diets that contained more than 14.9 g/kg Glyequi and 11.9 g/kg Glyequi, respectively.

Conclusions: These results suggest that a nutrient other than Glyequi limits growth when the CP concentration is reduced from 163 g/kg to 147 g/kg. Growth and N efficiency responses of broilers to dietary Glyequi are influenced by the CP concentration of the diet.

	Gly_{equi} (g/kg)	ADG (g/d)	ADFI (g/d)	G:F (g/g)	N efficiency (%)
132 g/kg CP	11.9	34.4^{g}	52.5^{e}	0.65^{g}	75.5^{ab}
	14.9	36.7^{f}	55.0^{de}	0.67^{f}	74.9^{b}
	17.9	38.9^{e}	56.5^{cd}	0.69^{e}	75.5^{b}
	20.9	42.3^{d}	59.0^{c}	0.72^{d}	76.6^{a}
147 g/kg CP	11.9	44.5^{c}	62.5^{b}	0.71^{d}	74.9^{b}
	14.9	46.9^{b}	64.7^{b}	0.73^{c}	74.9^{b}
	17.9	46.0^{bc}	63.1^{b}	0.73^{bc}	73.2^{c}
	20.9	45.8^{bc}	62.6^{b}	0.73^{bc}	72.0^{d}
163 g/kg CP	11.9	52.7^{a}	71.8^{a}	0.73^{b}	71.3^{d}
	14.9	53.5^{a}	71.3^{a}	0.75^{a}	69.9^{e}
	17.9	52.8^{a}	70.3^{a}	0.75^{a}	70.2^{e}
	20.9	52.6^{a}	69.6^{a}	0.76^{a}	69.3^{e}
Pooled SEM		0.74	0.96	0.003	0.39

$^{a-g}$ Different superscript letters within each column indicate significant differences ($P<0.05$).

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21.

Effect of a surplus dietary L-arginine supply on the nitrogen metabolism of restrictively fed growing cockerels

Einfluss einer diätetischen Argininüberversorgung auf den Stickstoffmetabolismus restriktiv gefütterter, wachsender Junghähne

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L-arginine (Arg) is dietary indispensable for chickens and contains the highest molecular proportion of nitrogen (N) among the proteinogenic amino acids. Moreover, Arg serves as pivotal precursor of multifunctional nitric oxides and cell proliferating polyamines affecting animals" N metabolism, feed intake and body growth. As stressful dietary conditions such as absolute and relative nutrient deficiencies can modify the metabolism of N, Arg and its metabolites, this study examined the effect of a surplus dietary Arg supply on the N metabolism of growing cockerels nutritionally stressed by moderate feed restriction. To exclude possibly interfering effects of surplus dietary Arg on avian feed intake restrictively fed cockerels were compared with *ad libitum* fed ones.

Methods: Thirty-two one-day-old Lohmann Dual cockerels were commercially reared in a floor-range system for 3 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 (CON): 1.37% Arg; Arg supplemented diet (ARG): 2.04% Arg; n=16 birds/diet). In addition, from day 28 to 49 both groups were subject to an *ad libitum* (AL) and restrictive (RES) feeding regime, respectively (n=8 birds/diet*regime). During the entire study the daily feed intake of RES fed birds corresponded to 75% of AL fed birds. After 7 days of dietary adaptation a N balance trial with two periods of 7 days each was carried out from day 35 to 49. In this trial birds were weighed weekly and residual feed was recorded daily. Twice a day, excrements of each bird were collected entirely and stored as weekly pool samples at -20°C. Feed samples and freeze-dried excrements were analysed for dry matter and Dumas N according to VDLUFA methods. Due to regime-dependent differences in live body weight (BW), balance parameters were related to birds" metabolic BW ($kg^{0.67}$). Beside the determination of birds" daily weight gain (DWG), N intake (DNI), N excretion (DNE) and N balance (DNB), the N efficiency ratio (NER; quotient of DWG and DNB) and N utilisation (NU; quotient of DNB and DNI) were calculated. At day 35, 42 and 49 blood samples were collected from wing veins of each bird to analyse serum levels of N metabolism associated parameters such as total protein, albumin, uric acid and urea via serum chemistry analyser (Eurolyser Vet CAA®). Statistical analysis was performed as 2 x 2 x 2 three-factorial ANOVA with „diet", „regime" and „week" as well as their interactions as fixed effects using SAS 9.4 procedure MIXED (2012) with repeated measures and Tukey-Kramer test. Differences between groups were considered as significant for $p \leq 0.05$.

Results: Resulting from feeding regime, AL fed birds showed 20% higher mean DWG (43.6±0.77 vs. 34.5±0.77; $p<0.001$), DNI, DNE and DNB than RES fed birds over both trial periods ($p<0.001$; Table). As both diets were isocaloric and isonitrogenous, mean DWG and DNI did not differ between CON and ARG fed birds. However, latter ones showed 9% higher mean DNE ($p=0.01$) and slightly lower NU than CON fed birds ($p<0.001$). Additionally, a minor diet-dependent difference in mean DNB was detected in AL fed cockerels only ($p<0.05$). Whereas feeding regime did not influence serum levels of examined blood parameters, mean serum urea level of ARG fed birds was 20% higher than in CON fed birds and correlated with mean DNE slightly ($r=0.248$; $p<0.05$).

Diet (D)	Regime (R)	DNI	DNE	DNB	NU	Urea
		g/$kg^{0.67}$/d	g/$kg^{0.67}$/d	g/$kg^{0.67}$/d	%	mmol/l
CON	AL	3.12	1.21	1.91 ^{a}	61.1	0.81
	RES	2.49	0.98	1.52 ^{c}	60.9	0.83
ARG	AL	3.14	1.35	1.80 ^{b}	57.1	1.03
	RES	2.53	1.06	1.48 ^{c}	58.5	0.99
PSEM		0.03	0.03	0.02	0.71	0.04
p values	D	0.298	0.01	<0.001	<0.001	<0.001
	R	<0.001	<0.001	<0.001	0.417	0.742
	D x R	0.761	0.366	<0.05	0.264	0.400

a,b,c: Values with different superscripts differ significantly within the same column ($p<0.05$).

Conclusions: The present study led to the conclusion that a sole surplus dietary Arg supply did not improve the N balance and subsequent body growth in moderate restrictively fed cockerels. On the contrary, the present results even indicated that the additional dietary Arg has mainly been metabolised to urea and renally excreted afterwards impairing the metabolic utilisation of dietary N.

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22.

Effect of partial dietary replacement of L-methionine by DL-2-hydroxy-4-methylthiobutyrate or DL-methionine on glutathione metabolism and oxidative stress in weaned pigs

Einfluss einer DL-2-Hydroxy-4-methylthiobutyrat- oder Methionin-Supplementierung in der Ration auf den quantitativen Glutathionstoffwechsel und oxidativen Stress bei Absetzferkeln

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Glutathione (GSH) is a major component of the antioxidant defense system. The rate-limiting amino acid for GSH synthesis is cysteine (Cys), which in turn is synthesized from methionine (Met) via homocysteine (Hcys). It has been suggested that transsulfuration (TS), the conversion of Hcys to Cys, and the antioxidant capacity in the intestine is increased when substituting dietary Met by the Met analogue DL-2-hydroxy-methylthiobutyrate (DL-HMTBA)1. However, we found earlier that TS rate is lower with a DL-HMTBA compared to an L-Met-supplemented diet2, which might result in a lower whole body GSH production. Therefore, we determined whole body GSH synthesis and the oxidative stress level of pigs fed a DL-HMTBA relative to an L-Met supplemented diet.

Material and Methods: Thirty one male, German Landrace pigs (8 ± 1.5 kg BW) were weaned on age day 28 and randomly allocated to a basal diet formulated to meet 70% of Met and Cys recommendation and supplemented on an equimolar basis with either L-Met (n=12), DL-Met (n=10) or DL-HMTBA (n=9) to provide 100% of Met and Cys requirements. On day 57 of age and after fitting with jugular and carotid catheters, fasted pigs received a primed, 8-h continuous intravenous infusion of [2H2]-glycine. Red blood cells (RBC) were isolated and [2H2]-glycine enrichment in GSH was analysed by GC-MS to calculate GSH fractional synthesis rate (FSR). Concentrations of RBC GSH, oxidized glutathione (GSSG), and the sum of GSH and GSSG (total GSH) were quantified by HPLC. The activity of RBC glutathione peroxidase (GPx) and plasma superoxide dismutase (SOD) as well as plasma concentrations of thiobarbituric acid reactive substances (TBARS) and derivatives of reactive oxygen metabolites (dROM) were determined using commercial kits. The effects between diets were analyzed by ANOVA using PROC MIXED of SAS.

Results: Whole body GSH FSR and RBC total GSH concentrations were lower in DL-HMTBA and DL-Met as compared to L-Met pigs (Table 1). The RBC GSH concentrations were lower in DL-Met compared to L-Met supplemented pigs. However, the GSH:GSSG ratio, GPx and SOD activities, as well as plasma TBARS and dROM concentrations were not different between Met sources ($P > 0.05$).

Table 1. Fasted glutathione fractional synthesis rate and red blood cell GSH and GSSG concentrations in 57 days old pigs fed diets supplemented with either L-Met, DL-Met or HMTBA starting at weaning (day 28)

	L-Met	DL-Met	DL-HMTBA
GSH FSR, %/d	63.8 ± 7.0 [a]	30.6 ± 6.6 [b]	33.6 ± 7.9 [b]
GSH, mM	1.85 ± 0.07 [a]	1.62 ± 0.07 [b]	1.64 ± 0.07 [a,b]
GSSG, µM	28.2 ± 13.0	48.6 ± 11.5	26.6 ± 13.7
Total GSH, mM	1.90 ± 0.07 [a]	1.74 ± 0.07 [b]	1.70 ± 0.07 [b]
GSH:GSSG (mM/mM)	234 ± 33	155 ± 29	256 ± 34

Least square means ± standard errors with different superscript letters within row are different between groups ($P < 0.05$).

Conclusion: The lower level of whole body TS described earlier2 is accompanied by reduced GSH FSR after feeding a DL-HMTBA relative to L-Met supplemented diet to weaned pigs. Despite reduced total GSH concentrations, dietary DL-HMTBA supplementation did not affect the level of oxidative stress markers, thus different Met sources had similar impact on oxidative stress.

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23.

Different levels of L-Glutamine affect the integrity and viability of the porcine intestinal cell line IPEC-J2, *In vitro*

Unterschiedliche Konzentrationen an L-Glutamin verändern die In vitro Integrität und Lebensfähigkeit der porzinen Zelllinie IPEC-J2

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L-Glutamine (GLN) is an important energy source for porcine enterocytes and immune cells. Dietary GLN supplementation in feed can improve intestinal health e.g. villi height, crypt depth, and proliferation, reduce gut atrophy, and improve barrier function. These may prevent inflammatory diseases and improve feed conversion in piglets (1,2). Since piglet enterocytes show high capacity for glutamine utilisation, we hypothesised that the absence or high overdose of glutamine in the environment would have a detrimental effect on the integrity and viability of the porcine intestinal cell line IPEC-J2, when assessed *In vitro*. **Methods:** The IPEC-J2 cells were grown in ThinCertTM cell culture inserts (polyethylene terephthalate capillary pore membranes; 0.4 µm pore size; Greiner BioOne) compatible with 6-well plates (Greiner BioOne). Cells at passage ~85 were seeded at a density of 5 x 10^5 cells/well. They were maintained in the plates during 7 days (37°C; 5% CO2) changing to fresh medium every second day, until they reached confluency, using the TEER value as indicator. The growth media consisted of glutamine free Dulbeccos modified Eagle medium (DMEM)/Hams-F12 supplemented with different concentrations of GLN as follows: none, 0.58 mM, 1.15 mM, 2.3 mM (considered a normal concentration), 11.5 mM, 23 mM. Transepithelial electrical resistance (TEER) was measured hourly between 1h and 6h and at 24h of incubation. Cell death was assessed after 24h of incubation using propidium iodide in flow cytometry. The TEER and cell viability measures were determined in triplicate in three independent experiments. Data were log transformed and analysed by One-Way ANOVA with post-hoc Bonferroni corrections. The statistical significance was considered at $P \leq 0.05$. **Results:** The integrity of the cells along the experiment was higher in the media containing 23 mM and 11.5 mM of GLN as compared to 2.3 mM, 1.15 mM, 0.58 mM or no GLN. On the other hand, the percentage of dead cells was significantly higher when 23 mM and 11.5 mM of GLN were used as compared to lower concentrations of GLN ($P \leq 0.05$). A higher cell death was noted for IPEC-J2 growing in normal concentration vs. no GLN in the media ($P \leq 0.05$). **Conclusions:** We demonstrate that high concentrations of GLN improve the cell integrity but at the same time decrease the cell viability, when assessed In vitro. A decrease in the cell viability when normal or high levels of GLN are available in the environment could be caused by an intensive cell metabolism and synthesis of metabolites which are toxic to the cells, and these need to be further assessed. A high TEER values which reflects good cell integrity, and at the same time an increased cell death are intriguing outcomes and should be explored in more depth.

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24.

Effects of grain species, genotype and starch quantity on the postprandial plasma amino acid response in horses

Einfluss von Getreideart, Getreidesorte und Stärkemenge der Mahlzeit auf postprandiale Reaktionen von Aminosäuren im Blutplasma von Pferden

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In horses, essential amino acids (EAA) from the feed must be absorbed until the end of the small intestine. However, experimental data on pre-caecal digestible (pcd) amino acid (AA) contents in feeds for horses are rarely available. Postprandial (ppr) responses of plasma AA (PAA) might be rough estimates for absorbed, but not immediately utilized (e.g. by the gut wall) AA from the feed, provided that energy and EAA requirements are met (1). The question was how PAA respond to cereal grains (CG) from different species (GS) and genotypes (GG) fed to achieve different starch intakes after roughage pre-feeding.

Methods: Six mares of 530 ± 56.0 kg bodyweight (BW) and 6 ± 2 years of age were fed meadow hay (1.5 kg/100 kg BW per day) and a meal either of crushed oats, crushed barley or cracked maize grains during consecutive trials. Four GG per GS were offered consecutively. CG meal sizes were varied in a cross-over design according to a starch quantity (SQ) of 1.0, 1.5 or 2.0 g/kg BW. At test days, 1 kg hay was offered 60 min before feeding of CG and blood was sampled from the jugular vein 0, 30, 60, 90, 120, 180, 240 and 300 min after the CG meal, which were 48 measurements per single treatment (i.e. 6 horses × 8 time points). Afterwards, remaining hay was offered. AA were analysed by ion exchange chromatography or HPLC. Contents of pcdAA in the feed were determined (1). Statistical analysis was performed with SAS 9.4 MIXED considering fixed GS, GG, SQ and time effects, interactions and a random animal effect at a significance level of $P<0.05$. Linear and quadratic correlations between AA intakes and basal (300 min ppr) and maximal PAA levels were analysed.

Results: Intakes of pcdEAA were highest in oats and lowest in maize, with specific GG differences. The supply of pcdLys and pcdMet+Cys (during maize feeding) and pcdThr (during barley and maize feeding) was deficient with minimal 0.77 of the requirement (1). GG×SQ, GS×SQ and GS×time interactions were largely significant ($P<0.05$). The ppr PAA concentrations had peaks mostly after 30 or 60 min. PAA concentrations measured at 300 min ppr seemed to better represent actual basal PAA levels and were used as basal. For most PAA, basal and maximal concentrations differed among oats, barley and maize genotypes ($P<0.05$) and were different to each other ($P<0.05$). Maximum levels of plasma Lys, Met, Thr and Trp, e.g., were up to 1.8-fold the basal levels. Linear correlations between AA intakes (grain meals) and basal PAA concentrations were mostly low ($r<0.47$), but occasionally up to $r=0.69$ ($P<0.05$). No evidence was found for curvilinear correlations. Significant correlations between AA intakes and maximal PAA concentrations did not exist.

Conclusion: Meals of CG led to a fast increase of PAA concentrations. Overall, no clear relation was found between AA intake and basal or maximal PAA. Both, GS, GG and SQ had significant effects on ppr PAA kinetics. More sophisticated characteristics of GG seem to have impact and should be investigated in the future. Longer-term effects of pure hay feeding on PAA levels need to be clarified and also how they contrast with the effects of mixed hay:grain meals.

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

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25.

Amino acid ratios in feather, feather-free and whole body protein of meat-type chickens as related to the recommended ideal dietary amino acid ratio

Aminosäurenverhältnisse im Protein von Federn, federfreiem Restkörper und Ganzkörper von Mastgeflügel in Beziehung zum idealen Aminosäuremuster im Futter

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Data about amino acid (AA) composition of modern genotype of growing chickens are scarce but provide basic information for factorial approaches in evaluating AA requirements. The study aimed to analyze the AA composition of both feather and feather-free body fraction and to evaluate their impact on whole body AA composition of an actual genotype depending on age and gender. Finally, AA composition data are related to the ideal AA ratio (IAAR) as derived from literature [1].

Methods: A total of 90 male and 90 female one day old chickens (Ross 308) was randomly allotted to 30 floor pens (5 birds/pen; 15 pens/gender) and reared under uniform management and feeding conditions [2]. Both starter (1-22d) and grower diet (22-36d) utilized an equal mixture of corn, wheat, soybean meal, soybean protein concentrate and crystalline AAs to achieve nutrient and energy supply according to current recommendations. At start and further on weekly, 15 birds per gender (3 pens of 5 birds) were selected and 24h fasted before quantitative de-feathering. Homogenized feather and feather-free body samples were analysed for N and AA contents according to standard procedures of VDLUFA. Two-way ANOVA (SPSS software package) connected with Tukey-test was utilized to identify significant differences between variables age and gender, respectively.

Results and Discussion: Summarized results (Table) are presented as means of AA ratios with lysine as the reference AA (Lys = 100). As expected, the observed AA ratios in both of analyzed body fractions are quite different. In the feather protein (FP) fraction, most of the indispensable AAs exceeded the ratio of Lys as reference AA for more than 1.5 to 3.5 times. However, lower ratios were observed for histidine, tryptophan and methionine, respectively. In contrast, the feather-free body protein (FFBP) fraction yielded lower ratios of indispensable AA, except for leucine. Regardless of the different AA composition in both of the fractions and according to the higher protein partitioning within the feather-free body (85-95% depending on age and gender [3]), the whole body protein (WBP) AA ratios were quite similar to fraction FFBP. In addition, significant age- and gender-specific differences between analysed fractions were observed [4]. Consequently, age-dependent displacements of individual AA ratios in the body of both male and female birds and their possible impact on dietary IAAR formulation have to be taken into account.

	Lys	**Leu**	**Cys**	**Arg**	**Val**	**Phe**	**Thr**	**Ile**	**Tyr**	**His**	**Trp**	**Met**
FP	100	346	315	314	264	214	207	199	153	45	36	24
FFBP	100	100	14	90	57	54	57	54	43	36	19	29
WBP	100	108	23	98	63	59	62	59	47	37	19	28
IAAR	100	110		105	80	66	66	69		34	16	40

Conclusions: The body AA composition of modern meat-type chickens during growth is not constant. According to the observed variation of AA ratios in the feather and featherless body protein fraction also depending on age period and gender, responses on AA composition of the whole body of growing chickens need consideration. These factors are of importance namely for AA requirement studies based on factorial approaches.

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26.

Investigations on the amounts of crude protein and amino acids of organically cultivated winter cereals (wheat, rye, and triticale)

Untersuchungen zum Rohprotein- und Aminosäurengehalt ökologisch erzeugter Wintergetreide (Weizen, Roggen und Triticale)

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Cereal grains are used as basic components of diets for monogastric animals. Thus, their composition is of great interest regarding optimized diet formulation. Since amino acids must not be supplemented in organic diets, information on crude protein and amino acid contents of single components become especially important. Hence, we investigated variations of the amounts of crude protein and amino acids in organically cultivated winter wheat, winter rye, and winter triticale grains.

Methods: We collected winter cereal grain samples from field trials of various organically managed experimental locations in Germany in three years (2011-2013). Samples of 106 winter wheat, 106 winter rye, and 107 winter triticale grains were ground to pass a 0.5 mm sieve and stored at 8°C. Crude protein content was analysed according to the VDLUFA method (Kjeldahl, N*6.25). Amino acids were analysed using HPLC. Contents of tryptophan were only analysed for 19 winter triticale and 25 winter rye samples.

We used R to perform Wilcoxon rank sum tests to compare crude protein and amino acid contents of the three cultivars. We further conducted Pearson correlation analyses to test if the amino acid composition of the crude protein varies depending on the crude protein content of each cultivar.

Results: We found wide variations of the crude protein and amino acid contents of all three winter cereal cultivars. Winter rye had significantly higher contents of lysine, methionine, cyst(e)ine, threonine, valine, alanine and asparagine than winter wheat and winter triticale. Winter triticale had intermediate contents of crude protein and most amino acids (Table).

Table: Mean content and standard deviation of crude protein (g/kg DM) and 18 amino acids (g/16g N)

Cultivar		CP	Lys	Met	Cys	Thr	Trp	His	Ile	Leu	Val	Phe	Tyr	Arg	Ala	Gly	Ser	Pro	Asp	Glu
Wheat	M	12.2	2.9	1.7	2.4	2.8	1.1	2.6	3.2	6.5	4.1	4.5	4.2	4.6	3.5	4.0	4.4	9.3	4.9	28.5
	SD	1.21	0.21	0.24	0.35	0.18	0.10	0.30	0.11	0.22	0.16	0.20	0.21	0.26	0.17	0.20	0.32	0.41	0.25	1.29
		c	a	a	a	a	a	a	a	b	a	a	c	a	a	a	b	c	a	c
	r		-0.7	-0.5	-0.5	-0.3	-0.3		-0.2	-0.3	-0.6			-0.5	-0.6	-0.5		0.4	-0.6	0.6
Rye	M	9.1	4.4	1.8	2.6	3.4	1.1	2.7	3.3	6.2	4.7	4.5	2.6	5.3	4.5	4.5	4.2	8.6	7.6	22.0
	SD	1.58	0.61	0.17	0.32	0.34	0.09	0.32	0.34	0.58	0.47	0.63	0.33	0.57	0.46	0.46	0.51	1.39	0.87	3.03
		a	c	b	b	c	a	b	a	a	c	a	a	b	c	b	a	a	c	a
	r		-0.8	-0.5	-0.5	-0.7	-0.9	-0.4	-0.4	-0.4	-0.5		-0.5	-0.7	-0.8	-0.7	-0.2	0.5	-0.8	0.4
Triti-	M	10.0	3.8	1.7	2.3	3.3	1.1	2.6	3.	6.6	4.5	4.5	2.8	5.2	4.3	4.4	4.5	9.0	6.6	24.8
cale	SD	1.52	0.46	0.17	0.26	0.26	0.09	0.22	0.36	0.48	0.29	0.33	0.20	0.36	0.33	0.34	0.39	0.80	0.51	2.56
		b	b	a	a	b	a	ab	a	b	b	a	b	b	b	b	c	b	b	b
	r		-0.8	-0.6	-0.3	-0.5	-0.5		-0.4		-0.4			-0.4	-0.6	-0.5	-0.2	0.4	-0.5	0.5

Significant differences between the cultivars are marked with different letters. Furthermore, the correlation coefficient (r) for each amino acid to crude protein is displayed in the table if correlation was statistically significant ($p<0.05$).

Pearson correlations indicate that an increase of the crude protein content affects the amino acid profile of the crude protein of all three cultivars. We observed a decrease of the amounts of lysine, the sulfur-containing amino acids, threonine, tryptophan, valine, arginine, alanine, glycine, and asparagine/aspartic acid while proline and glutamine/glutamic acid contents increased.

Conclusions: It is often mentioned that in organic farming crude protein contents of grains tend to be lower than in conventional farming. Our results indicate that lower crude protein contents might be associated with an altered amino acid profile that contains more essential amino acids.

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27.

Investigations on the role of pancreas in degradition of branched chain amino acids in the pig

Untersuchungen zur Rolle des Pankreas im Stoffwechsel der verzweigtkettigen Aminosäuren beim Schwein

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In addition to its role as an essential protein component, leucine (Leu) displays several other metabolic functions such as activation of protein synthesis. This property makes it an interesting amino acid (AA) for livestock production. However, Leu surplus stimulates the degradation of all three branched-chain amino acids (BCAA) via the branched-chain α-keto acid dehydrogenase complex (BCKDH), causing peripheral imbalances among Leu, valine (Val), and isoleucine (Ile) and depriving feed intake and growth performance in consequence (1). Since BCKDH is largely in the active state in hepatic tissue, the liver was considered as major site of BCAA oxidation so far (2). However, in a previous study (1), the tissue specific activity was higher in pancreatic compared to hepatic tissue. The current study aimed to investigate the effects of combined BCAA-overdosing on BCKDH activity in liver and pancreas of weaned piglets.

Methods: A total of 32 male and female piglets [(Landrace x Large White) x Pietrain] were weaned at the age of 21 days and randomly allocated into four different groups (n=8) without adaptation time. Piglets were fed *ad libitum* one of four mashed diets based on corn, barley and soybean meal, and supplemented with free AA to a constant Leu: Val: Ile ratio (100:70:53): 1) Con (15% CP, positive control), 2) T-150 (16% CP, BCAA provided at 150% of piglets‘ estimated requirement), 3) T-200 (16% CP, BCAA provided at 200% of piglets‘ estimated requirement), and 4) Hi-Con (17% CP, all essential AA and arginine provided at 150% of piglets‘ estimated requirement). During an experimental period of 14 days, performance was documented weekly. At day 15, the piglets were sacrificed to collect blood and tissue samples. To avoid differences in the amount of ingested AA before sampling, the pigs were fasted for 12 h and then received 180 g of feed 2.5 h prior to sacrifice. Plasma AA, plasma α-keto acids and serum ketone bodies were determined by HPLC and BCKDH activity was assayed spectrophotometrically (1). Data were analyzed by General Linear Model ANOVA, using SPSS Statistical Software (IBM SPSS Statistics Standard 20, Armonk, NY, USA). In case of significant effects ($p<0.05$), means of the groups were compared by Tukey-test.

Results: Piglets fed the Hi-Con diet had a significant higher feed-conversion-ratio after 14 d than control animals ($p<0.05$), whereas other performance parameters where not affected by dietary treatment. The plasma AA profile reflected the dietary AA composition: Increasing dietary AA provision increased the respective AA concentrations and concentrations of the BCAA-derived α-keto acids in blood plasma. The pancreatic BCKDH activity per g wet tissue exceeded the hepatic activity 2-fold and responded stronger to increasing AA. However, the total tissue activity was 3- to 10-fold higher in liver than in pancreas in all treatment groups due to higher tissue weight. In line with the significant elevated BCKDH activities in the Hi-Con group, the concentrations of serum β-hydroxybutyrate was highest in the same treatment group.

Conclusion: The significant greater hepatic and pancreatic BCKDH activity, along with elevated ketone body concentrations in blood serum of piglets, oversupplied with all essential AA and arginine, leads to the assumption that BCKDH might be involved in AA catabolism beyond the BCAA. Furthermore, the pancreas could be considered as main extra-hepatic tissue participating in BCAA degradation as previously reported for threonine (3). Particularly with regard to the increase of 611% in pancreatic BCKDH activity and only 199% increase in hepatic BCKDH activity in the Hi-Con treatment compared to the Con group, the pancreas seems to act as safeguard for AA clearance in case of disproportionate intakes.

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This study was supported by Ajinomoto Eurolysine S.A.S. (Paris, France) and Indukern S.A. (Barcelona, Spain).

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28.

Experimental study to test choice preference of diets with different protein levels depending on diet colour and water temperature in Nile tilapia

Wahlversuche mit Diäten unterschiedlicher Proteingehalte in Abhängigkeit von der Färbung der Mischfuttermittel und der Wassertemperatur bei Nil-Tilapien

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Fish as most animals, show „nutritional wisdom" and select among different feed sources to compose a balanced diet that meets their nutritional requirements. Dietary protein is an important aspect in achieving efficient fish production. Our objective was to test a choice feeding model for Nile tilapia as well as to analyse with this model the feeding preference/selection for a higher or a lower protein content in the diet depending on water temperature.

Methods: An experiment was conducted in Nile tilapia reared in twelve 200-L tanks for 63days. The study had a multifactorial design (2 temperatures×2 crude protein levels×2colour-combinations) with stocking rates of 12 fish (average wt 38g/fish) per tank. Two different diets were formulated to contain 30% (low) or 40% (high) crude protein. Fishes were fed for 2 h twice daily at 8:00 and 15:00 h.

Table 1. Experimental design depending on water temperature and colour code (A+B) for diets

	Low temperature (22-23°C); tanks (n=6)				High temperature(30°C); tanks (n=6)			
	colour code A; tanks (n=3)		colour code B; tanks (n=3)		colour code A; tanks (n=3)		colour code B; tanks (n=3)	
	High Cp	Low Cp	High Cp	Low Cp	High Cp	Low Cp	High Cp	Low CP
Diet colour	red	yellow	yellow	red	red	yellow	yellow	red

Feed intake was recorded on daily basis for the whole experimental period. Individual body weights were measured weekly. Body composition analysis of fish was done in one pooled sample per tank.

Results: The choice feeding model using two different colours as indicators for the level of protein worked. Only in week 4, there was an effect of the colour-code on feed choice. Colour code of diets had no effect on body weight development. Rearing fishes in a high water temperature resulted in significantly higher body weight beginning after two weeks up to the end of the trial. Animals reared in high water temperatures also showed a preference for the high protein diet. The water temperature did not lead to an altered body composition depending on DM and ash contents of fishes.

Table 2. Body weight and %-intake of high CP-diet in Nile tilapia in a choice feeding model

Time point/ week	Body weight [in g]				% Intake high CP-diet			
	colour code		water temperature		colour code		water temperature	
	A	B	low	high	A	B	low	high
Start	38.9±7.97	38.8±7.42	39.0±7.38	38.7±8.01				
1	41.0±8.31	40.2±7.48	39.4±7.36	41.9±8.29	28.4±2.39	30.1±2.84	27.4^{b}±1.51	31.1^{a}±2.23
2	42.0±8.22	42.5±7.93	39.9^{b}±7.42	44.8^{a}±8.03	44.2±2.87	41.3±3.73	41.5±4.10	44.0±2.63
3	44.2±8.19	43.5±8.64	40.4^{b}±7.45	47.8^{a}±7.67	42.7±4.76	36.9±4.59	36.5^{b}±4.03	43.1^{a}±4.72
4	45.0±9.13	44.5±9.09	40.8^{b}±7.45	50.3^{a}±8.26	38.8^{a}±4.83	28.7^{b}±4.15	30.3±5.83	37.2±6.29
5	46.3±10.2	46.3±10.1	41.1^{b}±7.50	54.3^{a}±8.32	35.1±4.80	34.9±3.72	32.5^{b}±2.64	37.5^{a}±3.88
6	48.8±10.8	46.6±11.2	41.6^{b}±7.49	57.7^{a}±8.10	36.8±3.96	39.6±3.26	37.1±5.07	39.4±1.46
7	50.5±11.7	46.8±13.1	41.9^{b}±7.51	61.7^{a}±8.83	31.3±5.16	32.2±2.45	29.7±3.93	33.8±2.80
8	51.5±13.6	48.8±13.8	42.3^{b}±7.51	65.5^{a}±8.82	28.6±4.31	28.0±1.93	26.0^{b}±2.05	30.7^{a}±2.25
9	52.2±14.7	51.6±15.9	43.0^{b}±7.49	70.3^{a}±9.31	27.1±3.27	27.1±2.54	27.7±2.34	26.5±3.29

a, b denote significant differences in the same row for each parameter ($p<0.05$)

Conclusion: Fish can select the diet among different ingredient composition in a model using a colour code regardless of the colours shade of diets. At high water temperatures, protein intake was also relatively higher, which may be due to the increased requirement for growth.

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29.

Influence of a constant lysine arginine ratio in gradually crude protein reduced diets on performance and foot pad health in broilers

Einfluss eines konstanten Lysin-Arginin Verhältnisses im Alleinfutter für Broiler bei stufenweisen reduzierten Proteingehalten auf die Leistung und die Fußballengesundheit

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The current discussion about resource-efficient animal production inevitably leads to the use of diets with lower crude protein levels. The content of essential amino acids should be kept constant, if possible. In particular, arginine is important because in uricotelic animals exogenous arginine is needed for the urea cycle. If the excretion of substances usually eliminated through the kidneys works optimally, reduced-protein diets often lead to better litter quality, which benefits the foot pad health. Supplementation of arginine to the optimal ratio is cost intensive, therefore it is necessary to prove again and again the effectiveness of the supplementation.The hypothesis in this experiment was that supplementation with essential amino acids including arginine in protein-reduced diets provides consistent performance with improved foot pad health.

Methods: In a four week feeding trial, 360 ROSS 308 broilers of both sexes were randomly subdivided into four feeding groups with six replicates each (15 animals per replicate). One group received a standard control diet (starter d 1-7, grower: d 8-14; finisher: d 15-35), the other groups specially prepared experimental diets. The control group (CP-C) was offered a complete diet with a common protein content found in practice (XP-% as fed; starter: 21.5, grower: 20.5; finisher: 20.0; lysine/arginine: 100/115). In the experimental diets, the arginine:lysine ratio was constant whereas the protein content was lowered in steps of 1.00 percent each (CP-1 to CP-3). The levels of essential amino acids were held on the same level due to the supplementation of single amino acids. The growth limiting amino acids as lysine, methionine, threonine, isoleucine, valine and arginine were therefore added to the experimental diets in higher proportions to equalize contents of these amino acids. External assessment of foot pads (FPD) was done weekly by a scoring according to Mayne (0 = normal skin; 7 = > half of foot pad necrotic). Performance parameters were recorded individually (body weight=BW). The statistical analysis (1-way-ANOVA for BW and FCR [REGWQ]; non-parametic for FPD) was performed using the Statistical Analysis System for Windows the SAS® Enterprise Guide®, version 9.3 (SAS Institute Inc. Cary, USA).

Results: At start of the trials the average body weight of chickens was comparable (C: 43.1 g; CP-1: 42.7 g; CP-2: 43.0 g; CP-3: 43.2 g). Feeding diet CP-2 led to higher body weights end of week 3 and week 4. There were no differences in FCR and FPD depending on dietary concept.

Table 1: Average body weight, Feed conversion ratio (FCR) and foot-pad scores (FPD)

Species	Group	Week 1	Week 2	Week 3	Week 4
Body weight [in g, end of week]	CP-C	216^{A}±19.8	535^{A}±58.1	989^{BC}±116	1589^{BC}±189
	CP-1	218^{A}±16.5	541^{A}±51.7	1004^{AB}±116	1632^{AB}±199
	CP-2	217^{A}±15.6	552^{A}±47.1	1034^{A}±106	1678^{A}±182
	CP-3	206^{B}±20.1	517^{B}±45.6	956^{C}±99.8	1548^{C}±175
FCR [kg/kg; Ø in week]	CP-C	0.957±0.014	1.167±0.026	1.363±0.043	1.489±0.042
	CP-1	0.952±0.034	1.186±0.026	1.342±0.026	1.475±0.025
	CP-2	0.996±0.044	1.165±0.009	1.335±0.046	1.494±0.032
	CP-3	1.024±0.072	1.200±0.042	1.389±0.047	1.517±0.026
FPD [end of week]	CP-C	0.04±0.13	0.46±0.38	0.94±0.20	0.97±0.15
	CP-1	0.03±0.12	0.43±0.37	1.02±0.23	0.97±0.12
	CP-2	0.05±0.17	0.38±0.37	1.04±0.23	0.97±0.12
	CP-3	0.03±0.12	0.45±0.38	0.99±0.28	1.01±0.12

$^{A, B, C}$ averages differ significantly within a column ($p < 0.05$)

Conclusion: The use of protein-reduced diets with a constant lysin/arginine ratio (100/115) throughout day 28 of fattening period has no negative effect on performance parameters. Although about 5% kaolin was added to finisher diets CP-3, the FCR did not differ. Foot pad healths was very good and unaffected until the end of the fourth week of fattening.

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30.

Response of piglets and fattening pigs 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 Absatzferkeln und Mastschweinen auf eine Aminosäurenoptimierung des Mischfutters bei 50% Austausch von Sojaextraktionsschrot durch Insektenmehl (Hermetia illucens) oder Mikroalgenmehl (Spirulina platensis)

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Alternative protein sources, such as insect or algae meal 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 a partly defatted larvae meal (HM) from the black soldier fly (*Hermetia illucens*) or the blue green algae *Spirulina platensis* (SM) in diets for piglets and fattening pigs. Both of the alternative protein sources are high in protein (60.8 resp. 58.8% in DM) and have a balanced amino acid (AA) composition. The study aimed to evaluate the effects of 50% replacement of SBM on zoo-technical data and apparent precaecal digestibility (apcD).

Methods: Immediately after weaning (21-day-old) 40 male castrated modern genotype piglets [PIC 408 x (Large White x Landrace)] were randomly allotted to 5 experimental diets. Piglets were individually housed in flat deck cages. Following an adaption period (14 days) the experimental period lasted 25 days (8.9±1.0kg initial BW). Feed was offered three times a day to prevent excessive losses, but water supply was held on free choice level. The control diet (main ingredients: wheat, barley and SBM) contained 28% SBM. The experimental diets replaced 50% of SBM by HM resp. SM under study. Both a basic level (Lys, Met, Thr in diets II, III) and an advanced level of AA fortification (higher level of Lys, Met, Thr in diets IV, V) according to (1) were investigated. After finishing the piglet trial, 4 piglets from each of the diets I-III were sampled for ileal chyme (intestinal section 150 cm before ileo-caecal valve) for apcD of CP and AAs (not all presented here) using TiO_2 as marker. According to an averaged BW, the remaining animals were utilized to continue the single feeding experiment up to 75kg BW making use of diets I, IV and V (n=8) with age dependent adaptation of dietary CP and energy concentration. 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 for both experimental sections are shown in the table. As compared to the control (I) diet IV yielded very similar ADG and FCR data both for piglets and growing pigs. FCR data with SM-based diets were generally impaired, AA fortification under study yielded only a very limited response. First data of apcD of the CP fraction and lysine support the expectation that diets with alternative proteins yield a high level of apcD as compared to the SBM diet.

Diets	I (Control) (n=8)	II (HM) (n=8)	III (SM) (n=8)	IV (HM+) (n=7)	V (SM+) (n=8)
ADG piglets (g/d)	521^{c} ±36	439^{ab} ±54	411^{a} ±60	509^{bc} ±32	442^{ab}±48
FCR (g/g)	1.40^{a} ±0.10	1.47^{ab} ±0.10	1.60^{b} ±0.13	1.39^{a} ±0.10	1.53^{ab} ±0.15
apcD of CP** (%)	69.9 ±10.9	79.3 ±1.8	76.2 ±1.2		
apcD of lysine ** (%)	80.2 ±6.0	89.4 ±2.4	84.6 ±0.3		
ADG fattening pigs (g/d)	966^{ab} ±55			999^{b} ±38	933^{a}± 31.2
FCR (g/g)	1.83^{ab} ±0.08			1.78^{a} ±0.07	1.89^{b} ±0.04

Means in the same column with different superscript letters differ significantly ($p \leq 0.05$) **According to outlier test Diet I: n=3, Diet II: n=4, Diet III: n=2

Conclusion: Both partly defatted meal of *Hermetia illucens* and algae meal of *Spirulina platensis* yielded appropriate zoo-technical results when 50% of the SBM was replaced by these proteins. Insect meal based diets were superior when an advanced level of AA supplementation was applied. It is indicated that both protein sources provide diets with a high precaecal digestibility of CP and AAs.

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31.

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

Einfluss unterschiedlicher Cysteingehalte im Alleinfutter auf die Leistung und Parameter der Hautgesundheit junger Puten

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Introduction: In modern poultry production foot pad dermatitis (FPD) is a special challenge regarding animal welfare. Litter moisture was identified as the main factor causing FPD (1). There are efforts to improve skin and foot pad health by supplementing S-containing amino acids. The aim of this study was to evaluate potential effects of different dietary cysteine (Cys) levels at adequate methionine (Met) supply on skin/foot pad health, feather growth and skin barrier function in turkeys.

Methods: 244 turkey poults (14 days old; BUT 6, ♀) were assigned randomly to 5 treatments (group 1-4: n=54, 3 subgroups; group 5: n=28, 2 subgroups). Feed and water were offered ad lib., the intake was measured daily on group basis. The pelleted corn-wheat-soybean meal based diets were calculated to contain 100% of recommended Met values and 90/100/120/130% of Cys recommendation (2). Group 5 was offered a diet with nearly natural Met content. To reach lower Cys levels 20% starch were included in the diets (challenge). Individual body weight and FPD-Scores (3) 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. Flufenamic acid (750 µg/ml) was used as donor fluid. After 24 hours the concentration in the acceptor chamber was analysed by HPLC to measure skin permeability. The length of one distinct feather (bastard wing/alula) was measured. Statistical analyses were done by using the SAS® software (t-test and Kruskal-Wallis-test).

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

Group (Cys level, %[1])	1 (90)	2 (100)	3 (120)	4 (130)	5 (natural)
Diet, in DM[2]					
XP, g/kg	251	247	252	256	254
Met, g/kg	7.56	7.49	7.50	7.48	4.38
Cys, g/kg (in %)	**3.84 (93.9)**	**4.09 (100)**	**4.81 (118)**	**5.33 (130)**	**3.94 (96.3)**
MJ ME/kg (calculated)	13.4	13.4	13.5	13.6	13.4
Body weight, d 42 (g)	1682^{a}±337	1672^{a}±260	1667^{a}±249	1622^{a}±264	1428^{b}±233
Feed Conversion Ratio	1.65	1.65	1.66	1.69	1.87
DM of "final litter[3]", %	53.9	59.0	58.5	55.0	68.1
FPD-Score[4], d 42	3.38^{a}±1.25	3.45^{a}±1.26	2.76^{b}±1.03	2.51^{b}±0.877	1.50^{c}±0.638
FFA in µg/ml, HPLC[5]	3.21^{a}±0.929	3.83^{a}±1.73	3.18^{a}±1.38	3.07^{a}±1.17	3.56^{a}±1.63
Feather length (mm)	31.0^{bc}±2.58	33.5^{a} ± 3.64	32.4^{ab}±2.42	32.6^{ab}±2.05	30.0^{c}±3.12

[1]intended, compared to standards; [2]analysed values; [3]mixture of bedding, excreta, feed remains, feathers at trials' end; [4]low values more favourable (0=healthy; 7= >50% of foot pad necrotic); [5]FFA=Flufenamic acid content in acceptor chamber; [a,b,c] indicate sign. diff. within a row ($p<0.05$)

Group 5 had lowest feather length and body weight. Skin permeability did not differ between groups.

Conclusion: Group 5 (natural content) boasted signs of dietary deficiency. Even small reduction (-6%) of the Cys content led to reduced feather growth. Cys had no effect on barrier function of the skin. Higher Cys levels allowed improvement of foot pad health even if the birds were housed on litter with relatively high moisture contents (groups 3+4). At high/recommended dietary Met levels (except group 5) there was no difference in the Feed Conversion Ratio (FCR) but in combined Met- and Cys deficiency lower body weight and poor FCR were evident. The best FPD values (group 5) are due to reduced feed and water intake resulting in lower amounts of excreta, thus leading to higher DM content in the litter. Recommended Met and Cys levels allow highest feather growth.

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32.

Protein quality of piglet diets with a high inclusion level of algae meal (*Spirulina platensis*) or insect meal (*Hermetia illucens*) by graded fortification of dietary amino acid supply

Beurteilung der Proteinqualität von Ferkelmischungen mit einem hohen Einsatzniveau von Algen- oder Insektenmehl bei gestufter Verbesserung des Aminosäurenangebotes

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Currently, alternative proteins like insect or algae meals are in special focus on animal nutrition. As part of the multidisciplinary project „Sustainability Transitions" the actual investigation aimed to evaluate protein quality parameters of piglet diets at high inclusion level of partly defatted *Hermetia* meal (HM) or *Spirulina* meal (SM) depending on the extent of amino acid (AA) supplementation.

Methods: N balance studies were conducted with sixteen male castrated modern genotype piglets [PIC 408 x (Large White x Landrace)] selected for equal body weights (25 kg) and divided into four experimental groups (n=4). Following 5d adaptation faeces and urine were collected in two consecutive quantitative collecting periods of 5d each. In experimental diets, the main ingredients were wheat, barley and HM or SM. Consequently, final diets contained 21% SM or HM in order to achieve the recommended CP level (19%) in the diets. Diets HM and SM were supplemented with Lys to achieve 80% of the recommended supply (1). Diets HM+AA and SM+AA were fortified with an extended AA supplementation (Lys, Met, Thr) according to the recommended ideal AA ratio (1). Faeces, urine 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 by standardized net protein utilization (NPU_{std}) according to (3). For this procedure, a standardized daily N intake (NI) of 3500mg/$BW_{kg}^{0.67}$was applied. Statistical analysis (one-way ANOVA, Tukey-test, Games-Howell-test) run by SPSS (Statistics 24).

Results: The daily N balance data were not significantly different between the diets under study (Table 1). However, the highest apparent CP-digestibility was observed with both of the HM diets (80.5% and 82.9%). Extended AA supplementation improved the dietary protein quality (NPU_{std}) of diet HM+AA significantly, but not in diet SM+AA. However, a numerical increase was also observed in diet SM+AA.

Diets	HM (n=7)	SM (n=8)	HM+AA (n=8)	SM+AA (n=8)
N intake [mg/$BW_{kg}^{0.67}$/d]	3675 ± 179	3666 ± 274	3645 ± 226	3361 ± 579
N balance [mg/$BW_{kg}^{0.67}$/d]	1870 ± 121	1867 ± 199	2182 ± 325	1777 ± 420
Apparent digestibility CP [%]	80.5^{ab} ± 1.8	77.1^{a} ± 2.7	82.9^{b} ± 3.2	77.7^{a} ± 2.7
NPU_{std} [%]	63.6^{a} ± 2.1	63.7^{a} ± 3.4	72.8^{b} ± 6.7	65.2^{ab} ± 6.6

Means (± SD); NPU_{std}= standardized net protein utilization (standardized daily N intake = 3500mg/$BW_{kg}^{0.67}$); different superscript letters reveal significant differences between diets ($p \leq 0.05$).

Conclusion: High inclusion level (21%) of partly defatted *Hermetia* meal or *Spirulina* powder in piglet diets was well accepted and yielded appropriate dietary protein quality, but further improved by an advanced level of AA supplementation. This improvement was superior in the insect meal based diet. Ongoing research will further optimize the dietary AA balance in piglet diets with high inclusion of alternative proteins.

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33.

Effect of differently conserved herbage on nitrogen efficiency in dairy cows

Einfluss von unterschiedlich konserviertem Grünfutter auf die Stickstoffeffizienz von Milchkühen

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Feeding maximum forage to ruminants involves less competition with human food resources. Forages derived from herbage are often high in rumen-degradable crude protein (CP), resulting in poor nitrogen (N) utilisation by ruminal microbes and N losses to the environment. However, the mode of conservation can influence the CP factions in forage (1) and, thereby, the potential N utilisation by the animal. The objective of the present study was to determine the effect of three types of forage preservation on CP fractions and N turnover in dairy cows.

Methods: In a replicated 3 × 3 Latin Square arrangement, 6 multiparous Holstein cows (milk yield: 23.5 ± 3.9 kg/d; 270.3 ± 7.2 d in milk) were randomly assigned to 3 treatments. Each experimental period lasted 21 d and included a 14-d adaptation and a 7-d data collection period. During the adaptation periods, cows were fed for ad libitum intake either silage (SI), ventilated hay (VH) or field-dried hay (FH) and received 300 g/d of a mineral mix. During the collection periods, cows received 95% of the intake of the adaptation period. The herbage for the forages was a 34-d regrowth harvested at the 30 August 2016 from a ley mainly composed of *Lolium perenne*, *Trifolium repens* and *T. pratense*. After 24 h of wilting one third of the forage was baled without additives at a dry matter (DM) content of 56% (SI). A further third of the forage, after 26 h on the field, was put on the ventilation at an average DM content of 68% (VH). The rest was harvested after 72 h drying on the field at 86% DM (FH). During the data collection period, feed intake, milk yield and milk composition were recorded daily and excreta were completely collected. On d 2 and 5 of each collection period, ruminal fluid via stomach tube and blood were sampled. The CP fractions were analysed according to Licitra et al. (2). Data were evaluated by analysis of variance with type of forage included as the main factor in the model.

Results: The contents of CP (g/kg DM) and net energy for lactation (NEL, MJ/kg DM) were highest in SI (207; 5.9) followed by VH (187; 5.5) and FH (176; 5.4). Milk yield (19.7 kg/d) as well as fat (4.93%) and protein (3.79%) percentage did not differ ($P>0.05$) among treatments. Cows receiving SI had a lower ($P<0.05$) DM intake (17.3 kg/d) than cows fed VH (19.2 kg/d), and cows fed FH (17.9 kg/d) were intermediate. Daily N intake was greater ($P<0.05$) for VH cows (581 g) compared to FH cows (509 g). The N intake of SI cows (560 g) did not differ from the other cows. The N excreted with milk tended to be higher with VH (123 g/d) compared to FH (113 g/d; $P=0.08$) and SI (112 g/d; $P=0.07$). Faecal N excretion was similar ($P>0.05$) for all treatments (177 g/d) whereas urinary N excretion tended to be higher ($P=0.06$) for cows fed SI (307 g/d) compared to cows fed FH (251 g/d). Cows fed VH (295 g/d) were intermediate. Treatments had no effect ($P>0.05$) on the N balance which was negative (-27.2 g/d) for all cows. The proportion of faecal N (% of N intake) was lower ($P<0.01$) for cows fed SI (30.0) and VH (31.4) compared to cows fed FH (35.3). The proportion of urinary N (52.0% of N intake) did not differ ($P>0.05$) among treatments. Ruminal ammonia concentration (mmol/L) was highest ($P<0.05$) for cows fed VH (8.2) and lowest for cows fed FH (7.0). SI cows (7.4) did not differ from the other cows. Plasma urea concentration (mmol/L) was higher ($P<0.01$) in VH (7.2) and SI (7.2) cows compared to FH (6.4) cows. Cows fed SI (370 mg/kg) or VH (351 mg/kg) had a higher content ($P<0.01$) of milk urea compared to cows fed FH (306 mg/kg).

Conclusion: Feeding only forage derived from herbage in late lactation resulted in high urinary N losses and an N deficiency in dairy cows. The absolute excretion of urinary N was highest when cows fed silage, which probably had a higher content of non-protein-N than the two other forages.

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34.

Do reduced IGF-1 levels modulate Vitamin D metabolism in young goats?

Modulieren verminderte IGF-1 Spiegel den Vitamin D Metabolismus bei wachsenden Ziegen?

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Feeding ruminants, a nitrogen (N) reduced diet is a common approach to reduce feeding costs and environmental pollution. Due to effective rumino-hepatic circulation, ruminants are able to recycle N more efficiently than monogastric species. Weight gain and development are not influenced by an N-reduced diet, however in previous studies it was found, that reduced dietary N intake caused massive changes in calcium (Ca) homeostasis in young goats (1,2). During an N-reduced feeding, blood Ca, calcitriol and insulin-like growth factor 1 (IGF-1) concentrations were decreased. IGF-1 is mainly synthesized by the liver and kidneys. It is essential for the hydroxylation of calcidiol to calcitriol. In blood, IGF-1 builds a complex with the acid labile subunit (ALS) and IGF binding proteins (IGFBPs) which serve as carrier proteins and extend the half-life of circulating IGF-1. Therefore, it was hypothesized that the modulation of IGF-1 levels is the link between reduced dietary N intake and Vitamin D metabolism in young goats.

Animals and Methods: Seventeen young male colored German goats (two months of age) were divided into two groups, receiving either an adequate or a reduced N supply. Both diets were isoenergetic and provided for 8 weeks. Ionised Ca concentrations were determined in whole blood samples using an ion-sensitive electrode and serum calcitriol levels were measured commercially by radioreceptor assay. Plasma IGF-1 concentrations were analysed by immunoradiometric assay. The mRNA expression of renal and hepatic IGF-1, ALS, hepatic 25-hydroxylase (CYP27A1), renal 1α-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24A1) were determined by *q*PCR. Protein expression of IGF binding proteins (IGFBPs) were determined by Western Blot analysis. Concentrations of growth hormone (GH) were analysed by ELISA. Data were analysed by unpaired Student‘s t-test.

Results: Ionised Ca concentrations and serum calcitriol levels were significantly diminished due to the N-reduced feeding. Plasma IGF-1 concentration decreased due to an N-reduced diet, too. Interestingly, IGF-1 mRNA expression decreased in the liver whereas the expression was enhanced in renal cortex in N-reduced fed goats. The mRNA expression of hepatic ALS was diminished in the N-reduced fed animals. Expression levels of hepatic CYP27A1 and renal CYP27B1 were reduced due to the N-reduced feeding while renal CYP24A1 expression increased. Protein expressions of IGFBP 2 and 3 increased in the group receiving the N-reduced diet. GH levels showed no alternation.

Conclusion: The decline in calcitriol concentrations during a dietary N reduction could have been mediated by diminished levels of hepatic IGF-1 that is essential for the synthesis of calcitriol. A decrease in renal CYP27B1 expression, which modulated renal calcitriol synthesis, might be due to diminished levels of IGF-1, because IGF-1 has a direct stimulatory effect on this enzyme in monogastric species. Diminished CYP27A1 expression that catalyses the synthesis of calcidiol might contribute to decreased levels of calcitriol. Moreover, increased CYP24A1 expression that initiates the depletion of calcitriol could conduce to reduced calcitriol levels, too. Diminished levels of ALS, that binds IGF-1 and hence increases their half-life, might contribute to alter IGF-1 levels. In summary, IGF-1 concentration might be modulated by an N reduced diet and therefore could be the link between reduced dietary N intake and Vitamin D metabolism in young goats.

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35.

Effects of insect meal from *Tenebrio Molitor L.* on the phospholipid composition and desaturation of fatty acids in the liver of obese rats

Effekte von Insektenmehl von Tenebrio Molitor L. auf die Phospholipidzusammensetzung und die Desaturierung von Fettsäuren in der Leber von fettleibigen Ratten

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Insect protein is an alternative source of protein which could be become more important in the nutrition of livestock and humans in future. It has been shown that protein from insect meal has a high biological value. We have recently observed that – besides its nutrition value – feeding of insect meal has profound effects on metabolism. In obese Zucker rats we observed that feeding insect meal as a source of protein causes a dramatic reduction of the concentrations of triacylglycerols (TAG) and cholesterol (Chol) in liver and plasma (1). Moreover, plasma lipidomic analysis indicated that feeding insect meal affects desaturation of fatty acids and metabolism of phospholipids (PL). In order to further understand the effect of insect meal on lipid metabolism, the present study investigated the hypothesis that insect meal influences amounts of individual phospholipids and their fatty acid composition in the liver which is the central tissue of lipid metabolism in the body. As in our previous study (1), we used obese Zucker rats as an animal model.

Methods: 24 male (*fa/fa*) Zucker rats, 10-11 weeks of age, were allotted into two groups: (I) a control group which received a diet with 20% casein as source of protein (C group), (II) an insect meal group in which the casein was replaced on an isonitrogenous base by insect meal [Product TMP-Y465, isolated from ground yellow mealworms (*Tenebrio molitor L.*), provided by Ynsect, Évry, France). Insect meal consisted of 71% of protein and contained additionally 10% fat and 11% fibre. The amount of fat and fatty acid composition were equalized between the two diets with individual mixtures of fat (soybean oil, safflower oil and flaxseed oil), and the amounts of fibre in the diets were adjusted by varying the amounts of cellulose and cornstarch. Diets were fed for four weeks. TAG and Chol concentrations in liver and plasma were determined by enzymatic kits, molecular species of phospholipids were analyzed by a lipidomic analysis, mRNA concentrations of desaturases were determined by qPCR. The statistical analysis was performed by one-way ANOVA using the Minitab Statistical Software (Rel. 13.0, Minitab Inc., State College, PA). Differences between groups were analyzed using the Fisher‘s multiple range test. Means were considered significant at $P < 0.05$.

Results: Feed intake and final body weights did not differ between the two groups of rats. In agreement with our recent study (1), concentrations of TAG and Chol in liver and plasma were strongly reduced in the insect meal group in comparison to the control group ($P < 0.05$). The total concentration of PL in the liver was not different between the two groups. There was however a shift in the phospholipid composition between the two groups of rats: rats fed insect meal had a higher concentration of phosphatidylethanolamine (PE) and lysophosphatidylethanolamine (LPE) and lower concentrations of phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylinositol (PI) and phosphatidylglycerol (PG) than control rats ($P < 0.05$). The concentrations of ceramides and sphingomyelin were not different between the two groups. The ratios of PC/PE, PC/PS, PC/PI, PC/SM were reduced in rats fed insect meal compared to control rats ($P < 0.05$). Rats fed the insect meal diet had lower concentrations of PC, PE and PI species with 4 to 7 double bonds and higher concentrations of PC, PE and PI species with 1 to 3 double bonds ($P < 0.05$), indicative of an impairment of Δ6- and Δ5-desaturation. Moreover, rats fed the insect meal had strongly reduced mRNA concentrations of *ELOVL5, ELOVL6* (encoding fatty acid elongases), *FADS1* and *FADS2* (encoding Δ6- and Δ5-desaturase) compared to the control group ($P < 0.05$).

Conclusion: Feeding of insect meal had profound effects on the phospholipid composition and the desaturation of fatty acids in the liver. It is likely that the alteration of the PL composition (particularly the changed PC/PE ratio) and the impaired desaturation of fatty acids contribute to the reduction of TAG and Chol concentrations in liver and plasma of the rats fed insect meal.

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36.

Antioxidative and metabolic responses in the jejunum of heat-stressed dairy cows

Antioxidative und metabolische Adaptation des Jejunums hitzegestresster Milchkühe

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Heat stress is a major environmental hazard for health of dairy cows. Changes in the gut integrity specifically altered tight junction connections and the subsequent increase in the permeability to luminal endotoxins appear to be critical for eliciting local immune responses and ultimately survival to a heat load. Endotoxins may also induce oxidative stress which in turn influences cellular metabolism. However, our knowledge of the oxidative stress response and the metabolic adaptation of the intestine during heat are woefully insufficient. Therefore, the objective of this study was to compare the effect of short-term heat stress and pair-feeding at thermoneutrality on the anti-oxidative defense machinery and the jejunal metabolism of lactating dairy cows.
Methods: Ten non-pregnant 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 6 days 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 of the daily mean 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 4 days of HS or PF, cows were slaughtered to obtain jejunum mucosa samples. Protein separation was performed by 1D gel electrophoresis. Protein bands between 50 and 90 kDa were sliced from the gel and analyzed by LC-MS/MS. Further jejunum mucosa scrapings were utilized to analyze mRNA abundances of superoxide dismutase 1 (SOD1), catalase (CAT) and glutathione peroxidase (GPX) relative to the reference genes hypoxanthine phosphoribosyltransferase 1 and ribosomal protein L0. Enzyme activities of CAT, alkaline phosphatase (ALP) and lysozyme were measured by commercial kits. Differences between HS and PF cows were analyzed using the Mann-Whitney-U test or Student´s t-test including the UNIVARIATE procedure of SAS (Version 9.4).
Results: The mRNA abundance of CAT tended to be 2.2-fold higher in HS than PF cows (P=0.095), while the abundances of SOD1 and GPX did not differ between groups. The ALP activity was 260% in HS relative to PF cows (P=0.05), whereas CAT and lysozyme activities were not different. Protein abundances of aldehyde dehydrogenase (1.7-fold; P=0.095), aldolase 1-epimerase (2.3-fold; P=0.031), fructose-bisphosphate aldolase B (2.1-fold; P=0.036), short-chain specific acyl-CoA dehydrogenase (1.7-fold; P=0.087), and mitochondrial aspartate aminotransferase (2.6-fold; P=0.071) were or tended to be 1.7 to 5-fold lower in HS than PF animals. Numerous proteins identified as mitochondrial very long-chain specific acyl-CoA dehydrogenase (3.6-fold; P=0.056) and cytosolic aminopeptidase (5.9-fold; P=0.095) were or tended to be 4 to 6-fold higher abundant in HS compared to PF cows.
Conclusion: Greater ALP activity may occur as a general response to an impaired gut health, and higher CAT expression during heat exposure suggests an activated antioxidant defense specifically in peroxisomes of the jejunum. These antioxidant responses were accompanied with lower protein expressions of enzymes involved in carbohydrate degradation, glycolysis and short-chain fatty acid utilization, but greater abundances of enzymes participating in long-chain fatty acid oxidation and protein degradation in HS relative to feed restricted cows. Regulation of these pathways in the jejunum support metabolic adaptation to heat stress.

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37.

Effect of hazel (*Corylus avellana*) leaves on methane and urinary nitrogen emissions by sheep

Effekt von Blättern der Hasel (Corylus avellana) auf die Emissionen von Methan und Harnstickstoff von Schafen

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Ruminant husbandry comes along with methane and nitrogen emissions, which are detrimental to the environment. Nutritional strategies are a means to mitigate these emissions. Supplementation of plant secondary compounds such as tannins can reduce both, methane and nitrogen emissions, whereas lipid supplementation to ruminant feed only reduces methane emissions. Evidence suggests that tannins inhibit the growth of methanogens. Hazel leaves have been found to be promising in mitigating methane and ammonia formation from rumen fermentation *In vitro* (1). Here we examined if hazel leaf supplementation is indeed efficient to reduce methane and nitrogen emissions in live animals. Further, we examined by using the tannin-binding compound polyethylenglycol (PEG) if the tannins present in the hazel leaves are responsible for the mode of action.

Methods: Four types of experimental pellets were produced using lucerne (*Medicago sativa*) and 0%, 30% or 60% hazel leaves, the latter with and without 4% PEG. The diets consisted of experimental pellets and grass hay at a ratio of 80:20 (DM). The level of hazel in the total diet was 0%, 25% and 50%. Hazel leaves contained 8% total phenols whereof 76% were tannins, with almost equal proportions of condensed and hydrolysable tannins. The diet amount offered was covering 1.6 times the recommended dry matter (DM) supply for maintenance requirements of adult female sheep. The crude protein content in the complete diets was ≥ 13.2% on a DM basis. In a 6×4 crossover design, six adult sheep were allocated to different sequences of four experimental diets in four subsequent 18-day periods. Each period was divided into 11 days of adaptation in individual pens and 7 days of complete collection of faeces and urine in metabolic crates, including 2 days of individual methane measurements in respiration chambers. The data were subjected to analysis of variance considering treatment and period as fixed effects and animal as random effect. Differences among means were considered significant at $p<0.05$.

Results: Including hazel leaves at dietary levels of 25% and 50% did not impair feed intake but fibre digestibility and at the high hazel level also organic matter digestibility. Methane emission was reduced by up to 25 to 33% when given per head per day, per unit of intake and per unit of organic matter digested. Urinary nitrogen excretion decreased by 34% and 73% with 25% and 50% hazel leaves. Addition of PEG prevented most of the effects of hazel leaves to be exhibited. This indicates that in particular the tannins present in the hazel leaves were responsible for the mode of action.

Conclusion: As a forage for ruminants, hazel leaves seem to have the potential to substantially mitigate methane and urinary nitrogen emissions. The substantial shift in nitrogen excretion from easily-volatile urine-N to faeces-N is highly favourable from an environmental perspective as gaseous N emissions and N leaching potentials are considerably reduced. Even high dietary hazel leaf proportions were ingested, but there is a need to compensate its relatively low digestibility with well digestible diet ingredients. Although high amounts of hazel leaves are required for broad scale application in ruminant nutrition, our findings widen the spectrum of nutritional strategies to mitigate adverse effects of ruminants on the environment.

This study was supported by the China Scholarship Council and by the ETH Zurich Research Grant (ETH-49 15-1).

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38.

Grazing biodiverse mountainous pastures with different slopes throughout summer season affects feeding behaviour and diet selection of dairy cows

Die Beweidung artenreicher Gebirgsweiden mit unterschiedlicher Hangneigung beeinflusst das Fressverhalten und die Futterselektion von Milchkühen

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In mountain dairy farms, optimisation of the alpine grazing system is a key to success. It has been demonstrated that such biodiverse pastures affect milk and cheese nutritional composition and sensory quality. On the other hand, it is known that grazing dairy cows select preferentially grasses and legumes among other species such as mature grasses and forbs, and have preferences for special spots on the pasture. Choices may vary according to specific needs of the animals, botanical species, vegetative stage, pasture topography or grazing management. The resulting selective behaviour of the animals could affect milk quality. The aim of the present study was, therefore, to characterise the evolution of feeding behaviour and diet preferences of dairy cows grazing mountainous pastures with high diversity through the summer season.

Methods: Three groups of twelve cows each composed of four Holstein (22.9±3.9 kg milk/d), four Montbéliardes (24.1±3.3 kg/d) and four Aosta Red Pied (14.7±2.3 kg/d) were monitored during 3 weeks in June (cycle 1 of grazing) and 1 week in late July (cycle 2). They had access to 0.3 ha/livestock unit without concentrate on 1) a grass-dominated pasture with low diversity (L) and 2) two mountainous pastures with a higher proportion of forbs and biodiversity (both about 23 species/m²) but with different slopes (A1: 22°; A2: 7°). The latter were vertically divided into three zones (Z1, Z2 and Z3 from 1066 to 1172 m a.s.l.). During two successive days in each of the 4 weeks, the behaviour of six cows per group was observed for 3 h after morning milking and before and after afternoon milking by scan-sampling in 5-min intervals. At the beginning of each week, the composition of the pastures was investigated by characterising the proportion of each bite type theoretically possible in the plot. Diet selection, defined as the proportion of a bite type in the diet relative to its proportion in the plot, was quantified using Jacob"s index of selectivity (IS). These indices range from -1 (aversion) to +1 (selection). Data were analysed with PROC MIXED with repeated measures in SAS.

Results: The proportion of plot bites containing grasses decreased between cycle 1 and 2: from 91 to 55% in A1, from 85 to 74% in A2 and from 94 to 87% in L. On A-pastures, they decreased in favour of forbs and dry materials, whereas on L-pasture legumes increased by 12%. Gradient of forbs increased and those from legumes decreased on A-pastures from Z1 to Z3. Grasses were always preferentially selected by cows with IS ranging from 0.37 to 0.82, except for the end of cycle 1 on A1 (IS = -0.12). Cows did not particularly select legumes on L-pasture. On both A-pastures, they selected legumes in cycle 1 but not in cycle 2 anymore. Cows on L-pasture showed an increasing aversion against forbs with every grazing cycle. On A1, they avoided forbs at the beginning of cycle 1 but selected them in the end and during cycle 2 (IS = -0.25 vs 0.10 and 0.23). On the contrary, cows on A2 pasture selected forbs directly from the start of cycle 1 (IS = 0.15), less in the end (-0.07) and again in cycle 2 (0.08). This was associated with the exploration of the different zones of the plots on the A-pastures changing from using mainly Z1 and Z2 in cycle 1 to progressively using also Z3 during cycle 2. On A1, cows visited preferentially Z2 (46% of their time) than Z3 (14%), the one richer in forbs. On the contrary, on A2 they spent 33% of their time in Z2 and 21% in Z3, easier to access on this plot due to the lower slope and a different shape.

Conclusion: Dairy cows showed a clear preference for grasses during the whole grazing season. On L-pasture, no selection for legumes or forbs was observed. On A-pastures however, cows selected legumes and forbs but differently in the season. Even if the species richness was similar, behaviour of the cows differed through the season as they were probably influenced by the slope and structural constraints of the plot. Cows grazed preferentially at the lower end of the slope and after exploitation of these parts used the entire pasture. This could lead to different qualities of the milk, which will be analysed next in the present experiment.

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39.

Measuring methane emissions of cows fed with different concentrate proportions making use of the GreenFeed system

Messung der Methanemissionen von Milchkühen bei unterschiedlichem Kraftfutteranteil mittels GreenFeed

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Up to 12% of energy intake in dairy cows might get lost due to methane emission (Johnson and Johnson, 1995). In order to reduce methane emissions, feeding factors influencing methane emissions must be identified. While respiration chambers deliver accurate results, they alter the routine of animals and therefore cannot be used long-term. A convenient alternative is the GreenFeed system (C-Lock Inc., Rapid City, SD, USA), in which animals are fed several times a day with concentrate. During these feeding times mass fluxes of carbon dioxide and methane are measured. Daily average methane emissions are estimated from these spot measurements. The GreenFeed system does not affect behavior of the animals and might be used for long-term trials with high numbers of animals. The aim of this experiment was to investigate the suitability of the GreenFeed system to measure methane emissions of cows fed with different concentrate proportions.

Methods: Twenty nine pluriparous German Holstein cows (284 ± 64 days in milk; means ± SD) were allocated to two groups either receiving 20% (on dry matter base; n=15; LC) or 50% (n=14; HC) concentrate proportion in the diet. Methane emissions were measured by a GreenFeed system for three continuous weeks. Cows were allowed to enter the GreenFeed five times daily for five minutes with a minimum time interval between visits of three hours. On each visit cows received 272 g of concentrate. The statistical analysis was performed using the MIXED procedure of the SAS software (9.4) for repeated measures with a compound symmetry structure. The factors in the model were group, time and the interaction between them. *P*-values < 0.05 were considered to indicate significant differences. Results are presented as LSMeans ± SEM.

Results: HC group had a higher dry matter intake (DMI; 20.29 ± 0.39 vs. 15.11 vs 0.42 kg/d; $P < 0.001$). Energy corrected milk (ECM) was influenced by a group*time interaction ($P < 0.001$; Fig. 1). Group ($P = 0.04$) and time ($P < 0.001$) influenced methane emissions. HC group had higher absolute methane emissions (369 ± 14 g/d vs. 327 ± 14 g/d; $P = 0.04$). Absolute methane emissions were increased in week 3 (367 ± 12 g/d; both groups) compared to week 1 (345 ± 11 g/d; $P < 0.001$) and week 2 (331 ± 11 g/d; $P < 0.001$). HC group had lower methane emissions per kg DMI (18.4 ± 0.8 g/kg vs. 23.6 ± 0.9 g/kg; $P < 0.001$). Methane emissions per kg ECM (Fig. 2) were influenced by a group*time interaction ($P < 0.001$).

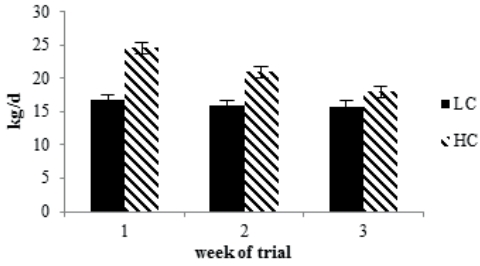

Fig.1 Energy corrected milk (LSMeans ± SEM)

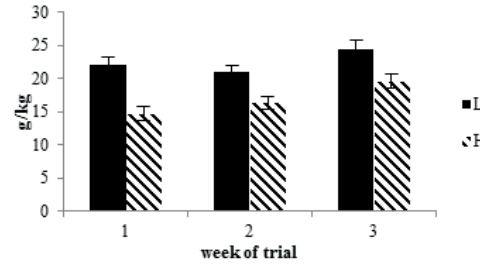

Fig.2 Ratios of methane emissions to ECM (LSMeans ± SEM)

Conclusion: The GreenFeed system was suitable to detect differences in methane emissions between groups fed with different concentrate proportions. While the HC group had higher absolute methane emissions, the ratios of methane emissions to kg ECM were lower for the HC group throughout the trial, which indicates a higher efficiency of cows fed with higher concentrate proportions.

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40.

Comparison of the quality of methane prediction equations containing a different selection and number of individual or groups of milk fatty acids as explanatory variables

Vergleich des Prognosewerts verschiedener Schätzgleichungen für die Methanemission unter Berücksichtigung individueller oder Gruppen von Milchfettsäuren

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The milk fatty acids (**MFA**) profile is a promising proxy for the estimation of methane production (**MP**) of cows. We investigated the effect of dietary composition and the number of explanatory variables (MFA) on the quality of MP prediction equations with the aim to identify MFA with the best predictive value.

Methods: Twenty half-sib German Holstein cows (106 ± 28 days in milk; 580 ± 57 kg BW), were fed four isoenergetic total mixed rations based on corn silage (**CS**), CS + linseed (**CSL**), grass silage (**GS**), and GS + linseed (**GSL**). Randomly selected cows (n=10) were fed CS and CSL diets for 5 weeks (wk) each (5 cows starting with CS), and the other ten cows were fed GS and GSL diets (5 cows starting with GS) for 5 wk each. 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 supplemented diets (GSL, CSL) contained 6% fat of DM compared to 3% fat in CS and GS diets. In wk 5, MP was measured in respiration chambers for 48 h and a milk aliquot was analysed for MFA concentrations by gas chromatography. Regression equations for MP (L/d) were obtained using PROC REG of SAS with the STEPWISE variable selection method. The complete data set included 46 different individual MFA. The limited MFA data set included sums of saturated (SFA), unsaturated (UFA), mono- (MUFA) and polyunsaturated (PUFA), C18:1 *trans* (*trans*6, *trans*9, *trans*10 and *trans*11), C18:1 *cis* (*cis9*, *cis*11 and *cis*12) and ω-3 MFA (C18:3 *cis*9, *cis*12, *cis*15, C20:5 ω-3, C22:5 ω-3), plus the individual C16:0 and C18:0 MFA. Regression equations were generated for MFA data from individual as well as all diets collectively.

Results: When using the complete MFA set to predict MP R^2 was between 0.97 and 1. The best model based on data from all diets contained C4:0, C6:0, C13:0, C15:0, C18:1 *cis*9, C18:1 *cis*11, C18:2 *cis*9, *cis*12, 20:2 ω-6, 20:3 ω-6, 20:5 ω-3 and C24:0. There was no discernible pattern of predictive MFA as explanatory variables in the regression equations based on the various diets. Using the limited MFA set to predict MP, R^2 ranged from 0.56 to 0.82. Combining MFA data from all dietary groups, R^2 was 0.59. Prediction equations resulting from the limited data set included C16:0 and C18:1 *trans* (GS), C18:1 *cis* (GSL), UFA, C18:1 *trans* and ω-3 MFA (CS), ω-3 MFA (CSL), PUFA, C18:0 and C18:1 *cis* (all diets) as explanatory MFA. There was also no discernible pattern of explanatory individual MFA or MFA groups in the regression equations.

Conclusion: Equations using 46 individual MFA produced excellent prediction of MP. Satisfactory prediction power was also obtained for the limited set of MFA. Thus, MP prediction from groups of MFA is possible. Using the complete MFA data set, the individual MFA C14:1 *cis*9, C15:0, C17:0 *iso*, C17:1 *cis*9, C18:1 *trans*11, C21:0 and C24:0 appeared in several of our equations. Others found that only C17:1 *cis*9 and C18:1 *cis*11 appeared in two or more published equations (1). A prediction equation based on all diets collectively provided satisfactory prediction with both data sets.

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41.

Investigations on the additivity of the effects of polyphenol rich plant extracts in methane mitigation *In vitro*

Untersuchungen zur Additivität der Effekte von polyphenolreichen Pflanzenextrakten in ihrer methanhemmende Wirkung In vitro

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There are various polyphenol-rich extracts for which a CH_4 mitigating potential has already been shown *In vitro* and *In vivo*. One example is the extract of the bark of *Acacia meransii* (AM) (1). Other examples are extracts from grape seed (GS) and green tea leaves (GT) (2). However, it is unknown whether effective extracts act additively, synergetically or antagonistically when provided in combination. The aim of this study was therefore, after confirming whether or not individual extracts have the potential to reduce ruminal CH4 production, to test if additive combination of extracts may be mutually efficient in mitigating CH_4 without concomitantly impairing ruminal fermentation.

Methods: The Hohenheim Gas Test was used to assess the effects of extracts of AM (CDM, Hamburg), GS (Zelang, China) and GT (Ajinomoto OmniChem, Belgium) and their combination on ruminal fermentation parameters *In vitro*. All extracts were incubated alone in a dosage of 50 mg/kg dry matter (DM). Furthermore, 50 mg/kg DM of AM was combined with 50 mg/kg DM of each of the two other extracts. Finally, 50 mg AM/kg DM was combined with both other extracts (25 + 25 mg/kg DM). The extracts were added to 200 mg DM of ryegrass hay and 30 ml of a rumen fluid buffer mixture (1:3) for 24 h at 39°C in 6 runs (n=6). Ryegrass alone served as a control. This resulted in seven treatments. After 24 h fermentation gas production was recorded and pH, NH_3 concentration, short chain fatty acids (SCFA) concentrations, as well as protozoal and bacterial counts of incubation fluid were analyzed. Concentration of CH_4 was determined in the fermentation gas. Data analysis was done with R. The lmer function was used to perform a mixed model with dietary treatment as fixed effect and run as random effect. Least square means were statistically compared with the control using Dunnetts test. Orthogonal polynomial contrast analysis was done for AM+GS and AM+GT versus AM+GS+GT to identify linear and non-linear (here: quadratic) relationships.

Results: Absolute CH_4 production and CH_4 concentration in fermentation gas were reduced (P<0.05) by all treatments, while AM alone lowered only CH_4 production but not CH_4 concentration. Supplementation of GS and GT alone did not reduce (P>0.05) gas production in contrast to all other types of supplementation. The NH_3 concentration in the incubation fluid was reduced (P<0.05) by all supplementation treatments except GT. There was no significant influence on pH, protozoal counts and total SCFA concentration of incubation fluid. Bacterial count was only increased (P<0.05) by GS while supplementation of GT extract alone increased (P<0.05) acetate concentration (mmol/l). The combination of AM+GT reduced (P<0.05) propionate concentration (mmol/l). There were various lowering effects on butyrate, iso-butyrate, valeriate and iso-valeriate concentrations with all extract combinations, but also with GT alone (on valeriate and isovaleriate). When comparing the combination AM+GS+GT with AM+GS and AM+GT, the relationship was linear for the reduction of total CH_4 and quadratic for the reduction in gas production.

Conclusion: The results show that especially GS is effective in reducing emission of CH_4 and NH_3 from ruminants without concomitantly affecting ruminal fermentation. GT seemed to support fiber degradation (increased acetate concentration) and reduced CH_4 emission without affecting fermentation negatively. Combining two extracts always had a negative influence on gas production, probably as the result of the twice as high total extract concentration rather than of an antagonistic effect. The same dosage but a combination of all three extracts depressed gas production further whereas the effects of this combinations on CH_4 emission was additive. Further *In vitro* and *In vivo* studies have to confirm that extract combinations tend to be less efficient in mitigation of CH_4 or more detrimental to fermentation than single extract types.

This study was supported by the Swiss National Science Foundation (Project no. 320030-149976).

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42.

Effect of flooring design (with/without litter/floor heating) in poultry (broiler/turkey) on development of body weight, feed conversion ratio and water to feed ratio

Einfluss der Bodengestaltung - mit/ohne Einstreu/Fußbodenheizung - in der Broiler- und Putenmast auf die Entwicklung von Körpermasse, den Futteraufwand und auf das Verhältnis von Wasser-/Futterverbrauch

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Housing of poultry on littered concrete floor in the EU is the common system for commercial poultry production. Litter quality itself has a significant influence on performance and health in the fattening of broilers and turkeys. The hypothesis of these experiments was that reducing the contact between birds and their excreta affects performance parameters as well as water intake in poultry.

Methods: Three consecutives trials with 240 chickens (Ross 308) each and five consecutives trials with 240 turkeys (Big 6) each were performed. After seven days animals were randomly assigned to four groups with three subgroups each. Different flooring designs were created: G1 – entire floor pens with litter, G2 – floor pens with litter and heating pad, G3 – partially (50:50) slatted flooring including an area that was littered, G4 – fully slatted flooring with a sand bath (900 cm2). Beside the two different drinking systems (nipple drinkers for broilers, bell drinkers for turkeys) housing systems were identical for both species. Number of animals was reduced by dissection at the end of day 21 to 144 animals in every trial. Feed and water intake was recorded daily on group basis. The development of body weight was carried out once a week at the end of the day. To compare the body weight, feed conversion ratio (FCR) and W: F ratio, One-way ANOVA (REGWQ) was used (SAS).

Results: Body weight showed no significant differences in both species before starting the trials. During the trial there were no differences in body weight in broilers whereas the average final BW at dissection (data shown before; [1]) in fully slatted flooring groups was significantly higher. For turkeys, the values for average body weight from day 21 onwards and the FCR in the group with fully slatted flooring were significantly higher. In both species water to feed ratio was significantly higher when using floor pens with litter and heating pad (average floor temperature in all trials: G1=27.0/G2=30.5/G3=26.5/G4=26.0 °C). The quality of the final litter was significantly worse in turkeys than in broilers (average DM in broiler trials: G1=71.2/ G2=75.1/G3-litter area=58.5% and in turkey trials: G1=46.1/G2=51.3/G3-litter area=45.8%).

Table 1: Average body weight (±SD; in g), feed conversion ratio (FCR) and water to feed ratio (W:F-ratio) in trials with broilers and turkeys

Species	Group	Average body weight [in g]					FCR	W:F-ratio
		d7	d14	d21	d28	d35	d8-35	d8-35
Broiler (n=720; n=429***)	G1	198±22.6	528±61.4	1067±127	1757±188	2479±269	1.48	1.85^{b}
	G2*	198±17.1	527±56.6	1060±130	1760±167	2486±247	1.46	2.02^{a}
	G3	197±20.7	535±51.4	1075±108	1779±163	2532±243	1.45	1.84^{b}
	G4**	198±19.3	540±51.5	1086±116	1790±148	2554±247	1.45	1.85^{b}
Turkey (n=1200; n=718***)	G1	168±17.1	370±43.6^{bc}	687±73.9^{c}	1197±97.8^{bc}	1894±161^{bc}	1.40^{b}	2.57^{b}
	G2	167±18.2	366±44.4^{c}	689±78.9^{bc}	1184±105^{c}	1866±182^{c}	1.41^{b}	2.85^{a}
	G3	169±18.0	376±44.6^{ab}	702±74.0^{b}	1218±98.1^{b}	1912±162^{b}	1.41^{b}	2.53^{b}
	G4*	168±18.0	379±47.6^{a}	720±84.0^{a}	1266±124^{a}	1969±161^{a}	1.44^{a}	2.48^{b}

G1: litter; G2: litter with heating pad; G3: partly - 50% - slatted flooring; G4: fully - 100% - slatted flooring)
a, b, c averages not sharing a common letter differ significantly within a column on species level ($p < 0.05$). *two animals died, **one animal died, ***number of animals after first dissection.

Conclusion: Minimizing the contact the contact between birds and poultry manure seems to favour the performance in fattening poultry. In turkey hens using fully slatted flooring system might foster body weight development during the whole rearing period. In both species only the heating pad led to an increased water intake. There was no effect of the housing system per se on water intake.

The project is supported by funds of the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

[1] Chuppava et al. (2017) Proc. Soc. Nutr. Physiol. Vol. 26:164

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43.

Detoxification of deoxynivalenol (DON)-contaminated maize with sodium sulfite (Na_2SO_3) and its impact on performance and mycotoxin plasma concentration in fattening pigs

Die Detoxifikation von Deoxynivalenol (DON)-kontaminiertem Mais mit Natriumsulfit (Na_2SO_3) und dessen Einfluss auf die Leistung und Plasmakonzentrationen von Mykotoxinen beim Mastschwein

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Deoxynivalenol (DON) is a *Fusarium*-derived mycotoxin common in cereals such as maize and often associated with lowered feed intake and performance as well as immunomodulatory effects in pigs. Various methods of mycotoxin-detoxification in feedstuffs are under investigation and so far the chemical detoxification of *Fusarium*-contaminated cereal grains with sodium sulfite, proven by the metabolisation of DON to its less-toxic sulfonated derivatives, so-called DON sulfonates (DONS) during this process, appears rather promising. The aim of this study was to examine the detoxification of DON-contaminated maize with sodium sulfite (SoS) and its impact on the performance of fattening pigs.

Material and Methods: Wet-preservation of uncontaminated (CON) and mainly with DON contaminated (DON) maize kernels (44.4 mg DON/kg maize) was conducted with either of three SoS-levels (0, 2.5, 5.0 g/kg maize), 15 g/kg maize propionic acid and 20% moisture content for 63-70 d and preserved maize was included at 10% in fatting diets (barley-wheat based), resulting in six different diets. Barrows (Bundeshybridzuchtprogramm, n=16/diet) with an initial body weight (BW) of 32.5 ± 3.4 kg were housed individually in floor pens and equally divided on those six diets (3-phased regimen): CON- (no SoS treatment), CON2.5 (2.5 g SoS/kg maize), CON5.0 (5.0 g SoS/kg maize), DON- (no SoS treatment), DON2.5 (2.5 g SoS/kg maize) and DON5.0 (5.0 g SoS/kg maize). During the 10 wk-fattening phase different performance parameters such as BW, average daily gain (ADG), average daily feed intake (ADFI) and feed:gain ratio (F:G ratio) were determined. Furthermore feed (every 14 d) and blood samples (week 10) were taken for DON and DONS analysis. Data were statistically analyzed with proc mixed (SAS 9.4) with main factors mycotoxin (DON vs. CONS), SoS-level (0, 2.5, 5.0 g/kg maize), their interaction and an adjusted Tukey-Kramer-test as *post-hoc* procedure ($p \leq 0.05$).

Results: DON concentration in diet DON- amounted to 4.3±0.34 mg/kg feed and was reduced to 3.0±0.12 mg/kg feed in diet DON2.5 and to 1.5±0.14 mg/kg feed in DON5.0. This was also reflected in plasma concentrations: in DON- pigs, mean plasma levels were determined at 21.2±6.4 ng DON/mL and decreased to 77% (DON2.5) and 52% (DON5.0) of the original value. In the two latter groups this coincided with a concurrent increase in DONS-levels. The two main factors showed a significant interaction for BW at the end of the trial as well as for ADG and ADFI averaged over the entire experimental period (Table 1). Pigs receiving diet DON- demonstrated the lowest performance, but this performance was recovered to control levels by SoS-treatment. However, final BW and ADG in pigs fed DON2.5 did not differ significantly from DON-, but did for group DON5.0, indicating a slightly better recovery of performance when using 5g SoS/kg maize in the wet-preservation process. This is also supported by the DON plasma concentrations. Performance of pigs fed the three CON-diets did not differ significantly from each other.

Main factors		**Performance parameters (LSmeans±SE)**				
DON	**SoS**	**group**	**BW[#] (kg)**	**ADG (kg/d)**	**ADFI (kg/d)**	**F:G ratio**
CON	0	CON-	121.38±1.99^{a}	1.24±0.03^{a}	3.30±0.06^{a}	2.68±0.05
CON	2.5	CON2.5	117.53±1.99^{ab}	1.20±0.03^{ab}	3.32±0.06^{a}	2.76±0.05
CON	5.0	CON5.0	118.05±2.15^{ab}	1.20±0.03^{ab}	3.28±0.07^{a}	2.73±0.06
DON	0	DON-	111.14±2.06^{b}	1.20±0.03^{b}	3.91±0.07^{b}	2.65±0.05
DON	2.5	DON2.5	118.00±1.99^{ab}	1.20±0.03^{ab}	3.25±0.06^{a}	2.71±0.05
DON	5.0	DON5.0	121.67±2.06^{a}	1.26±0.03^{a}	3.35±0.07^{a}	2.66±0.05
ANOVA (F-Test), p-values						
DON			0.223	0.203	**0.017**	0.277
SoS			0.224	0.104	**0.004**	0.362
DON*SoS			**0.003**	**0.002**	**0.002**	0.961

#BW: body weight at the end of trial (week 10)

Conclusion: Our results indicate that SoS wet-preservation of DON-contaminated maize successfully diminished DON-concentration in the feedstuff and this was also reflected in pigs" plasma concentrations upon feedstuff inclusion in the diet. The DON-related decrease in performance in group DON- was completely recovered to control levels in group DON5.0.

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44.

Effects of oral endotoxin administration in heat stressed broilers

Einfluss einer oralen Endotoxinverabreichung im Masthuhn unter Hitzestressbedingungen

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Heat stress plays an important role in livestock animals. The gastrointestinal tract is one of first organs, which is affected by heat stress. The disruption of the gut permeability due to heat stress is already described in several studies. Endotoxins (also called lipopolysaccharides (LPS)) are constituents of the outer membrane of Gram-negative bacteria. If the gut barrier is impaired, toxins e.g. endotoxins can enter the blood stream. Once they have entered the blood flow, they can induce an inflammation response. In order to evaluate the effect of heat stress in combination with an oral LPS administration, a broiler trial was performed to evaluate the endotoxin activity in the intestinal content. Furthermore, the endotoxin concentration in the blood as well as the expression of selected genes in the gastrointestinal tract and spleen have been assessed.

Methods: Thirty-two one day-old broilers were randomly assigned to four groups after an adaptation phase of 28 days at thermoneutral conditions. On day 29, half of the birds were kept under thermoneutral conditions (23 °C) and the other half of the birds, were kept under heat stress conditions (36 °C) for ten hours. After seven hours, LPS from *Escherichia coli* O55:B5 (2 mg/kg body weight) were orally administrated to half of the animals in the thermoneutral group and heat stress group. This refers to a 16-fold dosage of the daily intake of endotoxins via feed. Three hours later animals were sacrificed to collect blood, intestinal content, and tissue samples (using endotoxin-free equipment). The endotoxin activity (Endotoxin units) in the intestinal content of the duodenum, jejunum, and ileum was measured with the *Limulus amoebocyte* lysate assay. The total endotoxin concentration (based on the 3-OH C14:0 concentration) in the serum was assessed by HPLC-MS/MS. The expression of selected genes was analyzed in the spleen, duodenum, jejunum and ileum via real-time qPCR.

Results: The endotoxin activity was significantly increased in the digesta of the duodenum by 16-fold and in the jejunum by 9-fold in heat stress animals receiving LPS compared to thermoneutral group receiving no LPS ($P < 0.05$). Furthermore, a trend for an increased endotoxin activity in heat stress animals without LPS administration compared to thermoneutral animals without LPS administration ($P < 0.1$) was observed in the duodenum. No effect of any treatment was seen on the endotoxin activity in the ileum. In general, endotoxin activity was significantly lower in the duodenum compared to the ileum ($P < 0.05$). The endotoxin concentration in the serum was significantly increased by 5-fold in heat stress animals receiving LPS compared to thermoneutral group receiving no LPS ($P < 0.05$). There was no significant effect of LPS administration or heat stress alone on serum endotoxin concentration. Heat stress significantly increased the expression of IL1-beta in the spleen (2-fold) as well as the expression of the intestinal alkaline phosphatase (ALPI) in the duodenum (2-fold) and jejunum (2-fold) ($P < 0.05$). In addition, heat shock protein (HSP) 70 was significantly increased in the spleen (8-fold), duodenum (6-fold), jejunum (7-fold) and ileum (4-fold) in heat stressed animals ($P < 0.05$). Oral administration of LPS significantly increased the expression of IL1-beta and ALPI in the jejunum (2-fold; 3-fold) ($P < 0.05$). When LPS was administered to heat-stressed animals, the expression of IL1-beta and HSP70 in the spleen (26-fold; 70-fold) was significantly increased ($P < 0.05$) compared to thermoneutral animals. Furthermore, this treatment significantly increased the expression IL1-beta, ALPI, HSP70 and claudin-1 in the duodenum (7-fold; 4-fold; 72-fold; 3-fold), jejunum (3-fold; 6-fold; 60-fold; 3-fold), and ileum (4-fold; 5-fold; 49-fold; 2-fold) ($P < 0.05$).

Conclusion: The results of the study suggest that oral administration of LPS can even enhance the negative effects of heat stress. Increased endotoxin activity in the gut as well as an impaired gut barrier led to an increased endotoxin concentration in the serum. As a result, the expression of several genes, was drastically increased, when LPS was administered orally to broilers during heat stress.

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45.

Effects of lactational stage and conjugated linoleic acid supplementation on glucose metabolism during hyperglycemic clamps in dairy cows

Einfluss des Laktationsstadiums und einer Supplementierung von konjugierter Linolsäure auf den Glucosestoffwechsel während hyperglykämischer Clamps bei Milchkühen

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Supplementing conjugated linoleic acid (CLA) is supposed to spare glucose due to the milk fat depressing effect of the trans-10, cis-12 CLA isomer, and allows repartitioning nutrients despite an energy deficiency in early lactation. The objective of the present study was to investigate glucose turnover and insulin responses in dairy cows during late pregnancy and in early lactation performing hyperglycemic clamps. The hypothesis tested was that CLA supplemented cows require less exogenous glucose to achieve a hyperglycemic state indicating an elevated endogenous glucose supply.

Methods: Twenty-two multiparous pregnant Holstein dairy cows entering parity 3.4 ± 1.6 (mean ± SD) were investigated from week 3 before (a.p.) until week 4 after parturition (p.p.). Based on parity and milk yield in the previous lactation, cows were assigned to a group with CLA supplementation (CLA; n = 11; 7690 ± 1386 kg milk) and one without CLA supplementation (CON; n = 11; 7664 ± 1797 kg milk, mean ± SD). Cows were supplemented daily either with 70 g of lipid-encapsulated CLA (6.8 g t10,c12 and 6.6 g of c9,t11 CLA isomer) or 56 g of control fat. Two cows were removed from the evaluation because of refusing the CLA supplemented concentrate and health disorders. The final dataset included 20 cows (CLA: n = 9; CON: n = 11). We conducted 3 consecutive hyperglycemic clamps (HGC, 4 h duration each) in weeks -2, +2 and +4 relative to parturition in all animals by infusing intravenously a 40% glucose solution to reach a target plasma glucose concentration of 6 mmol/L. From week -3 up to week +4 relative to parturition, milk yield and dry matter intake (DMI) were recorded daily, while body weight (BW) and milk composition were obtained once weekly. Blood samples were taken weekly and every 30 min during the HGC. Plasma was analyzed for concentrations of glucose, fatty acids (FFA), beta-hydroxybutyrate (BHB), insulin, triglycerides (TG) and cholesterol (CHOL). Insulin sensitivity was assessed by calculation of the revised quantitative insulin sensitivity check index (RQUICKI). Weekly performance and metabolic data from CLA and CON were compared using the mixed models. The model included week, group and week × group interaction as fixed effects. The initial values of the respective parameters were included as covariate in the model to account for differences at the onset of the experiment. The repeated subject was the individual cow. Metabolic and endocrine responses between CLA and CON during the consecutive hyperglycemic clamps HGC-2, HGC+2 and HGC+4 were compared by using a mixed model with time point of HGC, group and the time point × group interaction as fixed effects and the individual cow as repeated subject. Significant effects were considered at $P<0.05$.

Results: The CLA supplementation did not affect performance and metabolic parameters except for BHB and cholesterol. Plasma concentrations of BHB were higher in CLA compared to CON and were highest in week +3 p.p. ($P<0.05$). Cholesterol concentration was higher in CLA as compared with CON ($P<0.05$). Furthermore, insulin concentrations tended to be higher and insulin sensitivity to be lower by trend in CLA compared to CON ($P<0.10$). During the HGC in early lactation, insulin response was lower and decrease of FFA and BHB greater compared with the HGC in week -2 ($P<0.05$) though glucose target concentration achieved during the steady state period was similar for all three HGC. No effects of CLA supplementation on HGC characteristics were detected.

Conclusions: Supplementation of CLA did not increase basal glucose concentrations in plasma, but by trend reduced insulin sensitivity and responsiveness of tissues. In contrast to earlier reports, not glucose in general is spared during CLA feeding but body reserves are preserved without restraining animals performance. Confirming recent assumptions, CLA effects on cholesterol and triglyceride concentrations indicated beneficial effects on hepatic lipid export contributing to an improved efficiency of prevailing metabolites in circulation.

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46.

Effects of body condition score and concentrate proportion on fat depot masses in dairy cows during the periparturient period and early lactation

Einfluss von Body Condition Score und Kraftfutteranteil auf Fettdepotsmassen von Milchkühen während Transitphase und Frühlaktation

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In early lactation, the conflict between decelerated increase of feed intake and high energy requirement induces a negative energy balance and mobilization of body fat reserves, which might lead to subclinical ketosis. The aim of this study was to investigate the influence of body condition score (**BCS**) and concentrate proportion in the diet (**C**) on the quantity of individual fat depots during the periparturient period and early lactation.
Methods: Sixty dairy cows were divided into four groups according to a 2 x 2 factorial experimental design with the factors BCS and C. Before calving, all animals were either a low or a high BCS group ($\mathbf{BCS_L}$ or $\mathbf{BCS_H}$). All cows received the same ration consisting of 80% silage and 20% concentrate (70% maize silage, 30% grass silage on a dry matter basis) until calving. After calving, each group was subdivided into a low and high concentrate sub-group, with rations containing 35% ($\mathbf{C_{35}}$) and up to 60 % concentrate ($\mathbf{C_{60}}$). For the latter, the concentrate proportion increased from 35% to 60% during the first three weeks post partum (**p.p.**). During the experimental period, the BCS was recorded weekly. At week (**wk**) 6 ante partum (**a.p.**), wk1 p.p., wk4 p.p., wk10 p.p. and wk17 p.p. ultrasonic measurements were performed to estimate the subcutaneous (**sa**t), retroperitoneal (**rat**), omental (**oat**) and mesenteric (**mat**) adipose tissue weights according to Raschka et al. (2016). The coefficient of determination for estimation of the different fat depots ranged from 0.83 to 0.95. Data were analyzed using the MIXED procedure of SAS Enterprise Guide 6.1.
Results: The average dry matter intake of the $\mathbf{C_{35}}$ groups was 20.8 kg, whereas the $\mathbf{C_{60}}$ groups had an average DMI of 22.6 kg, from wk 1 to wk 17 p.p. The proportion of concentrate in the ration did not influence the weights of the adipose tissue depots (p=0.307). The BCS and periparturient time sub-group, respectively, the fat depots in wk6 a.p. and 1 p.p. differed significantly from those in wk4, wk10 and wk17 p.p.. Fat depot weights of BCSL and BCSH animals differed significantly at any time point. There were no interactions between any factors.

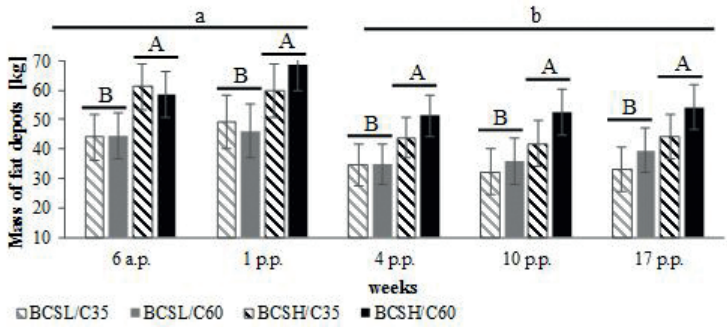

Figure 1: Total fat depot mass (sum of sat + rat + oat + mat) during the periparturient period and early lactation (LS Means), ^{a,b} indicate with $p < 0.001$ significant differences between weeks regardless of the different groups, ^{A,B} indicate with $p < 0.001$ significant differences between high and low BCS animals at each time point.

Conclusion: Different concentrate proportions in the rations did not affect the total fat depot mass in the periparturient period. The BCS before calving seems to be a key driver for fat depot mass changes during this period.

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47.

Effects of CLA supplementation on inflammatory and metabolic responses during an intramammary LPS challenge in early lactating dairy cows

Auswirkungen einer CLA-Supplementierung auf inflammatorische und metabolische Reaktionen während einer intra-mammären LPS-Challenge in frühlaktierenden Milchkühen

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The trans-10, cis-12 isomer of conjugated linoleic acid (CLA) causing a milk fat depression was shown to allow nutrient repartitioning despite an energy deficiency in early lactation and also to spare glucose. The „abundant" nutrients, in particular glucose, might improve the metabolic status and benefit the immune system. In the present study we investigated the potential nutrient sparing effects of CLA in early lactating dairy cows with low plasma glucose concentrations exposed to an intramammary LPS challenge. We hypothesized that CLA supplementation affects the metabolic and immune responses to LPS.

Methods: Fifteen multiparous Holstein dairy cows (parity: 2.8 ± 0.9, milk yield in previous lactation: 7,376 ± 1,247 kg, mean ± SD) were studied in week 4 after parturition (p.p.). Based on parity and milk yield in the previous lactation, cows were assigned to a group with CLA supplementation (CLA; n = 8) and one without CLA supplementation (CON; n = 7) 5 week before expected calving. Cows were supplemented daily either with 70 g of lipid-encapsulated CLA (6.8 g t10,c12 and 6.6 g of c9,t11 CLA isomer) or 56 g of control fat. In week 4 p.p., an intramammary LPS challenge (applied to one quarter) was performed in all cows as described earlier (1) to induce an inflammatory response. Milk (LPS and control quarter) and blood samples were taken every 30 and 60 min, resp.. In parallel to the blood samples, all cows were clinically examined. Plasma was analyzed for concentrations of glucose, free fatty acids (FFA), beta-hydroxybutyrate (BHB), cortisol, insulin, and glucagon. In milk, SCC and activity of lactate dehydrogenase (LDH) were determined. Performance and metabolic data were compared using the GLM procedure of SAS with group as fixed effect and initial values of the respective parameters as covariates. Metabolic, clinical, and endocrine responses of CLA and CON during the LPS challenge period were compared by using mixed models with time, group and the time × group interaction as fixed effects and the individual cow as repeated subject. Differences between CLA and CON groups were assessed by the Bonferroni corrected t-tests at $P<0.05$.

Results: In week 4 p.p., no differences in performance parameters were detected between CLA and CON, except with lower plasma glucose concentration in CLA ($P<0.05$). Between 2 and 3 h after LPS stimulation, rectal temperature, plasma cortisol concentration, LDH activity and SCC in milk began to rise. During the LPS stimulation, CLA tended to have higher rectal temperature compared with CON ($P=0.08$). However, the increase of body temperature in CLA started earlier, the difference between peak and basal temperature was higher, and the decline thereafter occurred earlier in CLA compared with CON ($P<0.05$). The increase from basal to peak glucose concentrations following LPS stimulation was higher in CLA compared with CON ($P<0.05$). Insulin concentration started to increase after 3 h relative to the LPS injection in both CLA and CON ($P=0.51$). Concomitant with glucose and insulin, plasma glucagon concentration increased. Whereas after 6h glucagon concentration started to decline again in CLA, it further increased in CON. During the first 2 h after LPS administration in each group, plasma BHB concentration remained similar within CLA and CON. Thereafter, BHB concentration declined to a higher extent in CLA and approached similar concentrations to CON from 5 h after LPS injection.

Conclusions: CLA supplementation of early lactating cows exposed to an intramammary LPS challenge affected local and systemic immune responses. Cows supplemented with CLA provided more glucose and preferentially used BHB as an energy source during the immune response. The more intense metabolic and more concentrated endocrine responses support an immunomodulatory effect of CLA supplementation.

1) WELLNITZ et al. 2011. J. Dairy Sci. 94: 5405-5412

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48.

Effect of feeding regime on serum metabolites in neonatal Holstein calves reared with milk replacer from day 3 to 14 *post natum*

Einfluss des Fütterungsregimes auf Serummetaboliten neonataler Holsteinkälber aufgezogen mit Milchaustauscher vom 3. bis 14. Tag post natum

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For economic reasons, the majority of established rearing protocols offer milk replacer (MR) to female dairy calves (FDCs) restrictively. However, during the early postnatal (*p. n.*) phase FDCs are highly sensitive to dietary limitations [1]. Consequently, FDCs require a sufficient dietary supply of energy and nutrients during their rearing to exploit their genetically determined long-term metabolic performance as mature dairy cows appropriately (metabolic programming, [2]). In this context, the present study examined variations in the short-term metabolic response of FDCs reared with two levels of MR from day 3 to 14 *p. n.*.

Methods: Twenty-four one-day-old female Holstein calves were *ad libitum* supplied with colostrum during the first two days *p. n.*. The calves were individually kept in straw-bedded calf hutches and received MR (200 g/L; 22.5% CP, 18.0 MJ ME/kg) *ad libitum* via an automatic calf feeder (AL; n = 12) or restrictively via teat buckets (RES; 3 x 2.5 L/d; n = 12) from day 3 to 14 *p. n.*. Water, hay and concentrate were offered from day 7 to 14 *p. n.* additionally. Individual MR consumption was recorded daily. At day 1, 7 and 14 *p. n.* calves were weighed and serum samples were taken from the jugular vein. Latter ones were analysed for clinical-chemical parameters of the energy, protein and lipid metabolism via spectrophotometry (ABX Pentra C400, Horiba Medical). Including a repeated statement for age statistical analysis was performed as two-factorial ANOVA (regime, age, regime x age interaction) with SAS 9.4 procedure MIXED (2012) at significance level $p \leq 0.05$.

Results: Daily colostrum intake did not differ between both groups (AL: 4.85 ± 1.50 L/d vs. RES: 4.96 ± 1.85 L/d) From day 3 to 14 *p. n.* daily energy intake was averagely 20% higher in AL than in RES calves (1.64 ± 0.07 vs. 1.34 ± 0.06 MJ ME/kg$^{0.75}$/d; $p < 0.05$), whereas calves daily weight gains did not differ between feeding regimes. The consumption of hay and concentrate was negligible. Among physiological, age-dependent alterations in the concentrations of examined serum metabolites (Table), AL calves differed from RES calves by age-independent 15 % lower serum urea concentration only ($p < 0.05$).

Table. Selected serum metabolites of female Holstein calves fed milk replacer *ad libitum* (AL) or restrictively (RES) in the first 2 weeks *p. n.* (LSMeans; PSEM; n = 12 calves/regime).

Metabolite	Feeding regime	Age (day *p. n.*)			PSEM	ANOVA (p values)		
		1	7	14		Regime (R)	Age (A)	R x A
Total protein (g/l)	**AL**	54.0	59.7	57.3	1.3	0.856	< 0.001	0.457
	RES	55.9	59.0	56.8				
Urea (mmol/l)	**AL**	2.80	2.99	2.63	0.28	< 0.05	0.192	0.435
	RES	2.98	3.94	3.09				
Glucose (mmol/l)	**AL**	5.62	8.37	6.33	0.44	0.648	< 0.001	0.163
	RES	6.31	7.38	6.10				
NEFA (µmol/l)	**AL**	523	172	222	42.1	0.502	< 0.001	0.305
	RES	438	175	253				
Triglycerides (mmol/l)	**AL**	0.21	0.43	0.30	0.05	0.173	< 0.01	0.434
	RES	0.21	0.31	0.29				

NEFA: Non-esterified fatty acids; PSEM: Pooled standard error of mean

Conclusions: In conclusion, the present study revealed that an additional energy and nutrients supply by 20% had only limited influence on the short-term metabolism of FDC in the early postnatal phase. However, the lower urea serum concentration in AL calves might result from a more efficient utilization of dietary protein in FDCs due to their absolute higher supply of dietary energy. As a consequence, such a short-term relief of early postnatal hepatic protein metabolism could be beneficial for the health of rearing calves [3] as well as the subsequent long-term metabolic performance of mature dairy cows [1,2].

[1] Kaske et al. (2010) Vlaams Diergen Tijds; [2] Metges et al. (2008): Proc Soc Nutr Physiol 17: 17-26; [3] Maccari et al. (2015) J Anim Physiol Anim Nutr 99: 737-746.

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49.

Effects of varying energy concentration of roughage and concentrate levels on feed intake and performance of Simmental cows over two years

Einfluss variiender Energiekonzentration des Grobfutters und unterschiedlicher Kraftfuttermengen auf die Futteraufnahme und Leistung von Kühen der Rasse Fleckvieh über zwei Jahre

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It is a well-known fact that industrial animal production has increased the demand for concentrates. These feeds are produced and transported at high environmental and economical costs.The aim of the study is to investigate different intensities in roughage and concentrates on performance parameters such as feed intake and milk yield in lactating dairy cows. The present work is part of the collaborative project *optiKuh*, supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

Methods: The experiment was conducted at the Bavarian State Research Centre for Agriculture over two years. 64 Simmental dairy cows, 1/3 of these were heifers, were randomly assigned to 4 treatments. Two different energy levels in roughage (6.1 = 6.1 MJ net-energy-lactation (NEL/kg DM) and 6.5 = 6.5 MJ NEL/kg DM) with two grades of concentrates (low N = 150 g/kg energy-corrected-milk (ECM) and high H = 250 g/kg ECM) were combined with a 2x2 factorial trial design (6.1N, 6.5N, 6.1H, 6.5H). Cows were offered a partly mixed ration and concentrates in automatic feeders according a fixed scheme in lactation. Feed intake and milk yield were measured daily and milk compositions were analyzed weekly. Based on feed intake and performance the energy balance was estimated according to recommendations of GfE (2001) [1]. Data were analyzed using the MIXED procedure of SAS (SAS Institute INC., Version 9.3). Roughage and concentrates and their 2-way interactions were considered as fixed effects in the model. The random statement included the cow within the treatment.

Results: In the group comparison, the ECM was significantly higher at 6.5H compared to 6.1N (Tab. 01). The energy intake followed the feed intake and the estimated energy balance (EB) showed an analogous pattern. While the estimated EB was positive across groups after approx. 7-9 weeks postpartum (p.p.), in the group with the lowest energy content (6.1N) it was in a positive range at a later time (about 13 weeks p.p.) and remains in further course of lactation slightly negative.

Table 1: Effects of concentrate level and the energy concentration in roughage on the characteristics of feed, milk and efficiency parameters during lactation (n = 16 cows per diet)

Item	Dietary treatment				p-value[1]				SEM[2]
	6.1N	**6.1H**	**6.5N**	**6.5H**	**Gr**	**R**	**C**	R*C	
ECM[3] [kg/day]	22.1^{a}	24.1^{ab}	23.0^{ab}	25.6^{b}	0.044	0.178	0.012	0.717	0.9
Feed intake [kg DM/day]	17.4^{a}	19.2^{b}	17.9^{a}	19.6^{b}	<0.001	0.157	<0.001	0.838	0.33
Roughage [kg DM/day]	13.8^{ab}	13.1^{a}	14.3^{b}	13.9^{ab}	0.019	0.020	0.043	0.630	0.28
Concentrates [kg DM/day]	3.7^{a}	6.2^{b}	3.7^{a}	5.8^{c}	<0.001	0.019	<0.001	0.016	0.09
Concentrates/ECM [g/kg ECM]	175^{a}	291^{b}	168^{a}	249^{b}	<0.001	0.0613	<0.001	0.163	13.4
Energy intake [MJ NEL/day]	112^{a}	128^{bc}	120^{b}	134^{cd}	<0.001	0.0004	<0.001	0.666	2.1

LS means within a row with different superscripted letters indicate statistically significant differences among the groups ($p<0.05$); [1]Gr: Group comparison, R: Roughage, C: Concentrates; [2]SEM, pooled standard error of mean; [3]Energy corrected milk calculated according to GfE (2001) [1];

Conclusion: In total it can be determined that there is a significant influence of the concentrate level on the milk yield as well as the feed intake but only a minor effect of the energy concentration of the roughage. With regard to the known relevant health effects associated with an insufficient supply of energy, the energy level in 6.1N is critical to see.

[1] GfE (2001). „Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder". DLG Verlag

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50.

Digestive plasticity of roe deer in response to changes in diet energy and diet quality

Flexible Anpassung des Rehs an unterschiedliche Energiekonzentration und Qualität natürlicher Nahrung

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The European roe deer (*Capreolus capreolus*) is a forest dweller and classified as a typical „Browser“ or „Concentrate Selector“ [1, 2]. This means that roe deer depend on a diet with a low fibre content and high proportion of crude protein[2]. This is why some hypothesize that roe deer are unable to digest agricultural plants, as these contain a relatively high proportion of fiber [3].

Methods: From 2011 to 2014, the rumen of 220 roe deer and data on the respective conditions were collected from legal hunts in both a forest habitat and in an agricultural habitat. In order to observe seasonal influences, we gathered samples throughout the 12 months of each year. We thus acquired a permit from the local hunting authorities to bag roe deer outside the regular hunting seasons. Our aim was to measure the quality and energy content of the natural roe deer diet (in terms of metabolizable energy (ME)) that we found in the rumen. Rumen contents were analyzed for crude nutrients including detergent fiber as well as for metabolizable energy (ME) according to standard methods and vitro fermentation (HFT), respectively. In order to get an overview of the total energy budget of the roe deer, we used a wildlife systems approach to analyze the condition and physiological adaption of roe deer to local and seasonal changes in diet. In addition, the availability and quality of the local vegetation and stress of the deer were evaluated as a measure of human disturbance.

Results: Rumen contents of roe deer from the agricultural habitat showed higher concentrations of ME in dry matter (DM) as compared to the forest habitat (6.4 vs. 5.4 MJ/kg, $p<0.01$) (Fig. 1). Inversely, rumen contents of roe deer from the forest habitat accounted on average for 300g more DM ($p<0.01$) than that of deer from the agricultural habitat. Thus, we found in both populations the same content of dietary energy of 1.5 MJ ME per rumen (when expressed as median over the year). Roe deer in the natural forest habitat thus compensate for the lower energy density of the vegetation with a higher rumen intake. Furthermore, in both habitats the proportion of crude fiber (XF) was not less than 21 % of dry matter and in winter, roe deer were able to extract more energy from the fibrous diet than the domestic grazer sheep.

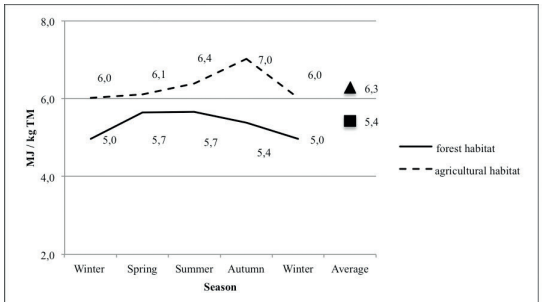

Figure 1: *Mean energy concentration (ME MJ / kg DM) in roe deer diet in two different habitats*

Conclusion: The German term „Concentrate selector“ should be replaced by „Selector“ or „Browser“ to avoid misinterpretation. During our study period, we did not observe any energy gap between the two populations. In neither of the two areas was supplemental feeding necessary for the roe deer to survive the winter. In terms of dietary energy, the roe deer in the agricultural habitat did not notice that it was winter. It might be hypothesized that the roe deer“s microbiome is adaptable to the local vegetation, permitting the animal to exploit energy from plants throughout the year and even with a high proportion of fiber.

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51.

New data on renal energy excretion in ponies eating various types of feed

Neue Daten zur renalen Energieaussscheidung beim Pony nach Aufnahme verschiedener Futtermittel

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To calculate metabolizable energy in horse feed from digestible energy a subtraction of 8 kJ/g protein is made regardless of the type of feed. In a recent study Hipp et al. (2017) formulated factors to predict renal energy excretion per gram of crude protein intake for different feed, which are based on protein content as a precursor of urea and content of available phenolic compounds such as ferulic acid, which are precursors of hippuric acid. Urea and hippuric acid are major drivers of renal energy excretion. Hipp et al. formulated a curvilinear predictive equation for gramineae products, with protein content in dry matter (x; g/kg DM) as independent and renal energy excretion (y, kJ/g crude protein) as dependent variable ($y = 0.0338x2-1.2539x +17.5091$; $r^2=0.64$; n=18, $p< 0.01$). In the present study we aimed to determine renal energy excretion of ponies eating different rations and to test the above mentioned predictive equation for various feed.

Animals, materials and methods: Four adult ponies (230-384 kg body weight) were available for the study. Six different diets were fed: hay first cut (HFC), hay second cut, and four combined diets with the same HFC and either fresh grass, clover-grass-mixture, sugar beet pulp and rice bran. Food allowance aimed for body weight maintenance, ratios of compounds were adapted according to expected tolerance. The trials lasted for at least 10 days. During the last six days of each experiment urine was collected completely (ponies were kept on a paddock with rubber mats, were they do not like to urinate, and brought into their stalls with sawdust in time intervals, where they urinated, and urine was collected with a bucket) alternatingly at night or during the day. Aliquots of urine were lyophilized for bomb calorimetry. Renal energy excretion per gram of crude protein intake was calculated for the total rations. In the combined diets the values for the compound which was added to the hay was calculated by difference.

Results: In hay of the first cut renal energy excretion amounted to 11.6 kJ/g crude protein and in the second cut hay as well as in the clover-grass mixture and the fresh grass it was much lower. By contrast rice bran and sugar beet pulp were similar to the first cut hay. When renal energy excretion was predicted according to the equation of Hipp et al. (2017) there was an excellent agreement ($r2=0.91$; $p<0.001$) between predicted and measured renal excretion.

Ration Hay or hay and test ingredient	% dry matter (DM)	Crude protein g/kg DM	Calculated renal energy losses from test ingredient kJ/g protein intake	Renal energy losses in total ration kJ/g protein intake
Hay, first cut (HFC)	100	66	-	11.6±3.6
fresh grass & HFC	52/48	130	4.5±1.2	6.5±1.0
Clover-grass-mix & HFC	52/48	103	7.8±1.5	8.6±0.9
Sugar beet pulp & HFC	35/65	71	10.6±5.0	11.3±1.9
Rice bran &HFC	33/67	76	10.3±0.8	11.2±0.6
Hay, second cut	100	116	-	7.8±0.8

Discussion: The data of Hipp et al. (2017) and of the present study show a promising method to predict renal energy losses. The highest renal energy losses are observed in HFC. The equation of Hipp et al. (2017) for gramineae products appears to be applicable to other forages including fresh grass and legumes as well as for high fibre concentrates such as rice bran. The distribution of all available data on protein content in DM and renal energy excretion in various horse feed, however, suggests a broken line model rather than a curvilinear equation for feed stuff > 16 % protein in DM. The rationale behind this is that the ratio of urea to hippuric acid excretion is determined by the ratio of crude protein to available phenolic acid content which is systematically affected by plant protein content in quite a number of feed.

Hipp et al. (2017) . J. Anim.Physiol. Anim. Nutr. , DOI: 10.1111/jpn.12756

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52.

Impact of dietary lignocellulose on animal performance and body composition of dual-purpose laying hens during the laying period

Einfluss der diätetischen Zulage von Lignozellulose auf die Leistung und Körpermassenzusammensetzung bei Zweinutzungshennen während der Legephase

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Dual-purpose hens have higher bodyweights and an increased body fat percentage in comparison with commercial hybrid hens (1). As there exists a strong negative correlation between bodyweight and reproductive efficiency (2), dual purpose laying hens show an inferior laying performance compared to commercial hybrids. Thus, the aim of this study was to investigate the effect of feeding an energy and nutrient-reduced diet containing lignocellulose on laying performance and body composition of dual purpose hens. It was hypothesized that nutrient-reduced diets decrease the bodyweight and body fat percentage of hens which might be accompanied with a higher laying performance.

Methods: One-day-old female Lohmann Dual chicks were allocated to 12 pens and fed two different diets for a period of 52 weeks: A standard control diet (C) and a treatment diet (LC), which was based on the control diet but supplemented with 10% lignocellulose (ARBOCEL®, J. Rettenmaier & Söhne GmbH + Co KG, Rosenberg, Germany). During the laying period (23 – 52 weeks of age) various performance parameters were recorded including the bodyweight (BW), feed intake (FI), egg weight (EW), egg production (EP), egg mass (EM) and feed efficiency (FE). At 52 weeks of age, hens were killed followed by analyses of the whole body composition (3). Data were analyzed by Students t test in SPSS (version 22.0, Chicago, IL).

Results: Results of the animal performance and body composition are displayed in the table below. Hens fed with LC had decreased bodyweights and an increased feed intake (Table 1). LC fed hens showed a higher egg production and egg mass resulting in an improved feed efficiency although egg weights were lower compared to hens fed C. Body composition analyses revealed that LC fed hens had higher protein concentrations and lower fat contents than hens fed C. Levels of crude ash and calcium were comparable between the feeding groups while the phosphorus content was increased in hens fed LC.

Hens Performance[1]	**C**	**LC**	**SEM**[2]	***P***	**Body composition**[1]	**C**	**LC**	**SEM**[2]	***P***
BW (g)	1996	1791	37.9	0.001	Dry matter (g/kg OS)	432	382	8.38	<0.001
FI (g)	101	107	0.71	<0.001	Crude protein (g/kg DM)	578	647	12.6	0.001
EW (g)	60.6	58.0	0.25	<0.001	Crude fat (g/kg DM)	384	259	20.4	<0.001
EP (%)	63.4	72.4	0.84	<0.001	Crude ash (g/kg DM)	82.8	87.2	1.46	0.133
EM (g)	38.1	41.8	0.47	<0.001	Calcium (g/kg DM)	23.7	25.0	0.65	0.333
FE (g feed/g egg mass)	2.87	2.61	0.04	0.001	Phosphorus (g/kg DM)	12.8	13.9	0.22	0.008

[1]Data are means of 6 replicate hens; [2] Standard error of the mean

Discussion and Conclusion: In comparison with dual purpose hens fed a standard layer diet, hens fed with dietary lignocellulose developed lower bodyweights despite of an increased feed intake. Layer performance was positively affected by the dietary inclusion of lignocellulose showing an increased egg production and egg mass accompanied with an improved feed efficiency although greater eggs were produced by hens fed with C. The body crude protein and phosphorus content was increased and the fat content was reduced in hens fed LC compared to those fed C. Thus, it can be assumed that the increased body fat percentage of C fed hens had a negative impact on the reproductive efficiency (2) as indicated by the lower layer performance compared to LC fed hens.

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53.

Effects of different energy supply from roughage and concentrates on signs of subclinical ketosis and subacute ruminal acidosis in early lactating cows

Auswirkungen einer unterschiedlichen Energieaufnahme durch Grob- und Kraftfutter auf Anzeichen von subklinischer Ketose sowie subakuter Pansenazidose bei frühlaktierenden Milchkühen

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At the beginning of lactation high yielding dairy cows exhibit a strong negative energy balance (**NEB**), which can result in subclinical ketosis (**SCK**). To attenuate this NEB, diets contain elevated portions of concentrates, increasing the risk to lower the ruminal pH and to evoke subacute ruminal acidosis (**SARA**). The aim of the study was to examine whether differences in energy supply from roughage and concentrates induce signs of SCK and SARA in pluriparous cows during early lactation.

Methods: This experiment was part of the joint research project optiKuh and comprised 63 German Holstein cows which were used from three weeks antepartum until 16 weeks postpartum (p.p.). During dry period all cows received an equal total mixed ration, whereas after calving they were assigned in a 2x2 factorial design. A partial mixed ration (**PMR**) consisting of grass and maize silage, concentrates and different portions of straw (18 % or 6 % of DM, respectively) was fed *ad libitum* and contained either 6.1 or 6.5 MJ NEL/kg dry matter. Additionally, concentrates were provided via an automatic feeding system to reach an amount of 150 or 250 g/kg energy corrected milk (**ECM**). Milk samples were taken twice a week to determine the milk composition. The fat-to-protein ratio (**F:P ratio**) was calculated for each animal and each week. A F:P ratio above 1.5 was considered as an indicator for SCK and a F:P ratio lower than 1.0 as sign for SARA, whereby animals exceeding these thresholds for at least two weeks during the trial were defined as suspicious (the first week of lactation was not included). Blood samples were taken on days -50, -14, 8, 28 and 100 relative to parturition and analyzed for β-hydroxybutyrate (**BHB**). SCK was defined for blood concentrations > 1.2 mmol/l. For assessment of clinical signs for SARA cows were clinically examined on days -14, 8, 28 and 100 relative to calving. The consistency of faeces and the occurrence of lameness were evaluated by scores. For evaluation of rumen function, the rumen contraction rate was determined and a scoring system was developed, in which rumen fill, ruminal tympany, intensity of ruminal contractions and the ruminal mat formation were reflected. Data were analyzed by using the MIXED procedure of SAS 9.4 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. Number of cows in groups being suspicious in F:P ratio or BHB concentration in blood were compared by using the Fishers Exact Test.

Results: The number of cows with SCK and with F:P ratio exceeding the thresholds is shown in Table 1. Cows with higher energy concentration in roughage had a slightly lower faecal manure score ($p = 0.026$), whereas the lameness score and rumen parameters were unaffected by treatment. For rumen health score higher values were observed 8 and 28 days p.p. ($p = 0.001$) independent of treatements, showing an impairment of rumen health during transition period.

Table 1: Number of cows being suspected for SCK or SARA during early lactation

Roughage, MJ NEL/kg DM	6.1		6.5		*p*-Value
Concentrate, g per kg ECM	150	250	150	250	(after Fisher's Exact Test)
Number of cows	16	16	16	15	
BHB > 1.2 mmol/l	5	4	3	7	0.405
F:P ratio > 1.5	9	7	9	9	0.810
F:P ratio < 1.0	2	6	0	3	0.030

Conclusion: Our results revealed slight signs of an elevated SARA risk by F:P ratio for cows receiving higher amounts of concentrates, but we consider that the findings of health assessment did not show clear negative impacts of the different experimental diets on animal health.

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54.

Varying the energy density of the diet by roughage composition and the amount of concentrates: effects on the circulating concentrations of adiponectin in Holstein and in Simmental cows

Einstellung unterschiedlicher Energiedichten in der Ration anhand der Grundfutterzusammensetzung und der Kraftfuttermenge: Effekte auf die Adiponectinkonzentrationen in Holstein- und Fleckviehkühen

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Adiponectin is a hormone produced by adipocytes and is presumably related to energy metabolism in dairy cows due to its insulin sensitizing effects. Our objective was to test whether the adiponectin concentration in serum of dairy cows in the first 100 days (d) in milk is influenced by different levels of energy in the diet and whether it makes a difference that the variation is achieved via feeding different amounts of concentrates or by changing the energy content in the roughage portion. The dataset presented is a subset from the national „optiKuh project“*.

Methods: From 3 experimental farms in Germany, 136 multiparous lactating dairy cows were allocated to receive either a roughage portion with 6.1 or 6.5 MJ NEL/kg DM ad libitum, combined with either 150 or 250 g concentrates/kg energy corrected milk yield (ECM). Thus 4 different feeding groups were obtained: 6.1_150; 6.1_250; 6.5_150, and 6.5_250. The lower energy in the roughage with 6.1 MJ NEL/kg DM was achieved by diluting with straw. To exclude possible carry-over effects, we only included cows in their first year of the trial. Farm A had 16 Holstein cows in each feeding group, Farm B had 6 Holstein cows in 6.1_150, 11 in the 6.1_250, 9 in the 6.5_150, and 7 in the 6.5_250 group and Farm C had 10 Simmental cows in the 6.1_150, 12 in the 6.1_250, 8 in the 6.5_150, and 9 in the 6.5_250 group. Individual feed intake and milk yield were recorded daily and milk composition was analyzed weekly. The energy balance (EB) was calculated on a weekly basis (1). Blood was sampled on d 8, 28, and 100 of lactation. The serum adiponectin concentrations were measured with an in-house developed bovine specific ELISA (2). For the statistical analyses the linear mixed model in SPSS 25 was used. There were no interactions with farm and thus a reduced model was used for the dependent variables adiponectin and EB, each (fixed effects: time, roughage, concentrate, farm, time x roughage, time x concentrate, roughage x concentrate, and time x roughage x concentrate; random effect: cow; Post-Hoc: Bonferroni). Level of significance was set to $p < 0.05$ and results are presented as means ± SEM.

Results: Adiponectin was neither affected by time, farm (and thus breed), nor concentrate, and there were also no interactions in this model. Cows receiving 6.1 MJ NEL/kg DM with the roughage had greater ($p = 0.043$) adiponectin concentrations (26.0 µg/mL ± 0.5) than cows fed roughage with 6.5 MJ NEL/kg DM (24.5 µg/mL ± 0.5). The EB was affected by time ($p < 0.001$) with a nadir on d 8 (-42.6 MJ NEL ±1.8) and highest levels on d 100 (13.4 MJ NEL ±1.7). Cows receiving 150 g concentrate/kg ECM had a more negative EB (-18.7 MJ NEL ±1.9) than those receiving 250 g/kg ECM (-9.6 MJ NEL ±1.8; $p < 0.001$). The EB also differed between farms ($p < 0.001$): Farm B (-1.01 MJ NEL ±2.6) > Farm A (-21.0 MJ NEL ± 1.8) and C (-20.6 MJ NEL ±2.4), whereas the energy in the roughage portion yielded no effects on EB. There was no correlation (Spearman) between adiponectin and EB. Until now studies were limited to test the effect of the energy level in the feed or energy balance on adiponectin in dairy cows, whereas the effect of different diet composition was not yet examined. Human studies have already shown associations between food composition and adiponectin concentrations, e.g. higher fiber content in the diet and higher adiponectin levels (3).

Conclusion: Our findings suggest that also in early lactating dairy cows serum adiponectin levels might be regulated rather by feed composition than by energy intake or balance in general. Associations of adiponectin with other metabolic parameters, i.e. β-hydroxybutyrate, non-esterified fatty acids and insulin have to be investigated in further analyses.

The study is part of the optiKuh project funded by the German Federal Ministry of Food and Agriculture (BMEL).

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55.

Effects of intensive milk feeding and butyrate supplementation on sucking behaviour, health and immune status in German Holstein calves

Einfluss einer intensiven Milchfütterung und eines Buttersäurezusatzes auf das Tränkeverhalten, die Gesundheit und den Immunstatus bei Kälbern der Rasse Deutsche Holstein

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The preweaning calf period is critical with regard to feeding management, growth development and vulnerability for diseases. Previous studies have shown that an elevated milk feeding intensity during the first weeks of life plays an important role for growth and development in preweaning calves (1). Furthermore, butyric acid (BA) is known to stimulate intestinal maturation and health (2). In the present study we have tested the hypothesis that intensive milk replacer (MR) feeding together with BA supplementation avoids detrimental feeding behaviour and supports the immune status and health of calves before weaning.

Methods: Holstein calves (n=64; half male and half female) were studied from birth until wk 11 of life. All calves received 2.5 kg of first colostrum from their dams, respectively. 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 or without 0.24 % butyrate, resulting in 4 treatment groups. Gradually weaning took place from wk 9 to 10 of age, whereas 2 L/d of MR were offered until the end of trial. MR was provided by an automatic feeding system and calves had free access to water, hay and concentrate. Feed intake was measured daily and body weight was determined weekly. Data for unrewarded visits and sucking rate were provided by the automatic milk feeding system. Blood samples for analysing immunoglobulin (Ig) IgG1, IgG2 and IgM, fibrinogen, haptoglobin and serum amyloid A (SAA) were taken after birth, on d 2, 4 and 7, then weekly until wk 11. Body temperature was measured daily for the first 3 wk and faeces, naval and respiratory health were scored daily. Performance data, body temperature and measurements in blood plasma were analysed by the Mixed procedure of SAS and health parameters were evaluated by a general linear model using the GLIMMIX procedure of SAS both models with feeding regimen, butyrate supplementation, time, and respective interactions as fixed effects.

Results: Intensive MR feeding resulted in a higher daily milk and MR intake ($P < 0.001$), higher MR intake per meal ($P < 0.001$), a slower sucking rate ($P < 0.001$) and a higher body weight gain ($P < 0.001$), but in a lower number of unrewarded visits at the automatic milk feeder ($P < 0.001$) and lower concentrate intake ($P < 0.001$) when compared to restrictive MR feeding. BA supplement reduced sucking rate ($P < 0.001$) but increased MR intake per meal ($P < 0.05$). Ig concentrations increased ($P < 0.001$) after colostrum feeding in all calves with a slightly greater concentration of IgG1 on d 4 and 28 in Res than Adlib calves and a slightly greater concentrations of IgM on d 1, 4 and 10 in calves fed BA. Plasma fibrinogen and SAA increased in first wk of life in all calves and fibrinogen was higher in Res than in Adlib on d 21, 49 and 63 ($P < 0.05$). Body temperature was greater ($P < 0.001$) in Adlib than in Res during the first 2 wk of life. Adlib calves showed a slightly greater rate of pasty and smooth faeces, but neither milk feeding intensity nor BA supplementation affected the health status.

Conclusions: Intensive milk feeding and BA supplement did not influence the health of the calves. The higher fibrinogen concentration in Res calves may indicate a greater threat for chronic inflammation in Res than in Adlib calves. Intensive milk feeding reduced the number of unrewarded visits and sucking rate, showing less detrimental feeding behaviour in ad libitum MR-fed calves.

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56.

Effect of diet and rearing on jejunal mucosal development, microbiota colonization and immune reactions in neonatal piglets

Einfluss von Diät und Umwelt auf die jejunale Mukosaentwicklung, mikrobielle Besiedlung und Immunreaktion in neugeborenen Ferkeln

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Hyperproliferacy in pigs increased the use of artificial rearing systems with formula feeding thereby influencing early-life gut colonization, likely with long-term effect on physiological and immune development. Here, we studied the influence of artificial rearing and formula feeding in neonatal piglets on small intestinal mucosal function, microbial colonization and immune reaction.

Methods: A total of n=48 new-born piglets (male, female) were randomly allocated into four different groups (n=12 each) in a 2 x 2 arrangement with environment (sow-reared (Sow) or artificial rearing unit/ isolator (Iso)) and diet (sow milk (SM) or formula (FO)) as main factors. Formula was based on skimmed milk powder and whey (22.6% CP, 20.0% EE, 46.0% lactose per kg DM). At days 7 and 14 of life, n=6 piglets per group were euthanized for sampling of blood, digesta and jejunal tissue. Jejunal cross-sections were analysed for morphology, proliferation (Ki-67) and intraepithelial lymphocyte (IEL) abundance ($CD3^+$). In addition, gene expression of the proliferation marker *PCNA* was determined. The IEL and peripheral blood mononuclear cell (PBMC) subsets were further characterized by flow cytometry using the following antibodies and their combinations: CD2, CD3, CD4, CD5, CD8, CD16, CD21, γδTCR1). PBMC subsets were futher characterized for expression of the transcription factors *Tbet* and *Foxp3* and pro-inflammatory cytokines (TNFα, IFNγ) using intracellular staining methods. Serum cytokines (i.e. IFNγ, IL-1β, IL-4, IL-6, IL-8, IL-10, IL12p40, TNFα) and lipopolysaccharide (LPS) were determined by ELISA. Mucosal scrapings were used for DNA extraction and illumina sequencing of 16S rRNA gene amplicons. Sequences were analysed using the MG-RAST platform. Data were evaluated using generalized linear models with environment and diet as main factors in SPSS. Analysis of bacteria and their relation to host-associated parameters was performed using SIMCA-P, CANOCO and R.

Results: Rearing environment and diet were associated with morphological changes ($P<0.05$). A higher *PCNA* gene expression in formula-fed piglets after 14 days was correlated with histological appearance of Ki-67 (R=0.39) and deeper crypts (R=0.54). Microbiota analysis revealed environment and diet-specific differences at phylum, genus (e.g. *Lactobacillus, Fusobacterium, Clostridium, Veillonella, Prevotella, Actinobacillus*) and species level and lower bacterial diversity in isolator-reared piglets. Analysis of jejunal IELs showed a lower abundance of $CD2^+CD3^-CD5^-$ cells in isolator-reared groups, and an increase of $CD8\beta^+$ T cells at day 14 in the Iso-FO group ($P<0.05$). In blood, total $CD4^+$, $CD8\alpha^+$ and CD4/CD8 dp cells did not differ, but a lower abundance of $CD2^+CD3^-CD5^-$ cells and $\gamma\delta TCR1^+$ cells was observed on day 7 in isolator-reared piglets ($P<0.05$). Lineage differentiation markers revealed a higher abundance of *Tbet* expressing $CD4^+$, and CD4/CD8 dp and $\gamma\delta TCR1^+$ cells in sow-reared piglets at both time points, indicating enhanced Th_1-cell polarization likely due to higher exposure to a more diverse microbiota. Similarly, a higher abundance of TNFα expressing $\gamma\delta TCR1^+$ cells was found, whereas no differences were observed for IFNγ expressing subsets. No significant differences were found for *Foxp3* expressing cells. Finally, serum LPS and pro-inflammatory cytokines (e.g. IFNγ, TNFα, IL-12p40) were higher in sow-reared piglets after 7 days, whereas an opposite effect for LPS was observed after 14 days ($P<0.05$).

Conclusions: Isolator-rearing has strong influence on microbiota composition, epithelial and systemic immune reaction in neonatal piglets, with some effects being further amplified by formula feeding. The data suggest an early pro-inflammatory stimulation with normal natural bacterial colonization in sow-reared piglets. Whether a delayed colonization and Th_1 response in isolator-reared neonatal piglets is associated with long-term health effects warrants further studies.

This study was supported by the German Research Foundation (Pi 946/2-1).

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57.

Effects of cinnamaldehyde on electrophysiological parameters of the porcine colon *In vitro*

Effekte von Cinnamaldehyd auf elektrophysiologische Parameter am Colon des Schweins In vitro

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Introduction: Phytonutrients are being investigated for their potential to improve the efficiency of nutrient digestion in animal feed. Previous studies have shown that the addition of cinnamaldehyde influences ion transport across the intestinal epithelium of rodents and pigs [1, 2, 3], but both a stimulation of anion secretion and cation absorption have been reported.

Material & Methods: The colon of freshly killed young pigs was rinsed, stripped, transported to the laboratory in ice-cold buffer solution and subsequently equilibrated for 45 minutes in Ussing chambers at 37°C. Throughout, tissues were gassed with 95% O_2 / 5% CO_2. Changes in short circuit current (Isc) and conductance (Gt) were used to monitor effects of adding 1 mmol·l^{-1} cinnamaldehyde after replacement of an ion or addition of a blocker. Control tissues were from the same animal and treated with the vehicle or a sham solution change before application of cinnamaldehyde. For data evaluation, the changes in Isc and Gt were calculated 15 minutes after the ion replacement or blocker, yielding ΔI_{base} and ΔG_{base}, and 15 minutes after addition of cinnamaldehyde, yielding ΔI_{cinn} and ΔG_{cinn}. Statistical comparisons were performed using the paired or unpaired rank sum test, as appropriate.

Results: In standard NaCl buffer solution, mean Isc was 9.10 ± 2.12 µA·cm^{-2} and mean Gt 20.34 ± 0.75 mS·cm^{-2}. Addition of 1 mmol·l^{-1} cinnamaldehyde led to significant increases by ΔI_{cinn} = 18.35 ± 2.15 µA·cm^{-2} and ΔG_{cinn} = 4.72 ± 0.35 mS·cm^{-2} (both $p < 0.001$, n/N = 54/10). To test for neuronal involvement, lidocaine (1 mmol·l^{-1}) was applied, which induced a significant change in ΔI_{base} versus control ($p = 0.002$, n/N = 15/3), but showed no effect on ΔG_{base}, ΔI_{cinn} or ΔG_{cinn} (all $p > 0.7$). Chloride secretion was inhibited by low chloride solution (9.8 mmol·l^{-1}, both sides, n/N = 30/4), NPPB (chloride channel blocker, 0.5 mmol·l^{-1}, n/N= 21/5), the NKCC blocker bumetanide (1 mmol·l^{-1}, n/N = 16/3), or the prostaglandin synthase inhibitor indometacin (0.01 mmol·l^{-1}, n/N = 9/3). All treatments led to a significant decrease in ΔI_{base} ($p < 0.05$). NPPB, which can also be expected to interfere with anion exchange, additionally induced a significant rise in ΔG_{base} ($p < 0.001$). However, low chloride solution or bumetanide had no significant effect on ΔI_{cinn} ($p > 0.1$), while effects of indometacin and NPPB were only partial (62% and 47%, $p < 0.001$). Of these interventions, only low chloride had a small effect on ΔG_{cinn}, which was reduced by 25% ($p = 0.037$). Conversely, bilateral replacement of Na^+ by $NMDG^+$ (n/N = 17/3) significantly reduced not only ΔI_{base}, ΔG_{base} ($p < 0.001$) but also ΔI_{cinn} and ΔG_{cinn} (by 86% and 79%, both $p < 0.001$), probably reflecting both effects on Na^+ transport and inhibition of basolateral NKCC. Replacement of mucosal Na^+ reversed Isc, most likely reflecting paracellular serosal to mucosal flux of Na^+. ΔI_{base}, ΔG_{base} and ΔG_{cinn} all dropped ($p < 0.001$, n/N = 28/4), while ΔI_{cinn} remained unchanged. The ENaC blocker amiloride (1 mmol·l^{-1}) significantly reduced the baseline parameters ΔI_{base} and ΔG_{base} ($p < 0.05$, n/N = 15/3) but showed no effect on the cinnamaldehyde response (ΔI_{cinn} or ΔG_{cinn}). Conversely, the non-selective cation channel blocker quinidine (1 mmol·l^{-1}) significantly reduced ΔI_{base}, ΔG_{cinn} (both $p < 0.001$, n/N = 9/2) and ΔG_{base} (0.048). Replacement of mucosal Ca^{2+} by EGTA (1 mmol·l^{-1}) increased ΔI_{base}, ΔG_{cinn} and ΔG_{base} ($p < 0.002$, n/N=6/2). Numerically, ΔI_{cinn} dropped in response to quinidine and rose in response to EGTA, most likely reflecting simultaneous changes of Na^+ absorption and K^+ secretion through the pore of a non-selective cation channel.

Conclusion: The data suggest that roughly half of the cinnamaldehyde response is related to cAMP dependent chloride secretion, most likely via NKCC and CFTR. Quinidine sensitive, amiloride insensitive cation channels may represent the other half of the conductance. More research is necessary to elucidate these effects.

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Funding: Akademie für Tiergesundheit (D. Manneck)

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58.

Characterization of an inducible amino acid transporter in the porcine gastrointestinal tract

Charakterisierung eines induzierbaren Aminosäuretransporters im Magen-Darm-Trakt des Schweines

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Methionine (Met) as an essential and as one of the first limiting amino acids is an important feed additive for poultry and swine. A previous study demonstrated that a diet containing DL-Met could induce a Na^+-dependent transporter for L-Met in the porcine small intestine (1). In the present project this amino acid transporter was functionally characterized.

Methods: Ten weeks old piglets were pre-fed with a diet supplemented with 0.21% DL-Met. To assess Met absorption, the apical uptake of ^{14}C-labelled L-Met (U_{Met}) was measured over 1 min in jejunal sections. Tissues were mounted in Ussing chambers to ensure a strict separation between the mucosal and the serosal side. The effects of Na^+, Cl^- and different inhibitors on Met uptake were examined at a mucosal L-Met concentration of 50 µM. Data were compared by two- or one-factor ANOVA with post-hoc Student-Newman-Keuls´ test as appropriate. In further experiments, uptake kinetics of the induced transporter were determined by measuring U_{Met} in presence and absence of Na^+ at increasing mucosal L-Met concentrations ranging from 5 µM up to 2 mM. Data were fitted to the Michaelis-Menten equation.

Results: Baseline U_{Met} at 50 µM concentration amounted to 904 ± 192 pmol·cm^{-2}·min^{-1} in presence of Na^+ and Cl^-. In a two-factorial design, the omission of Na^+ or the omission of both Na^+ and Cl^- equally decreased U_{Met} to 65.1 ± 6.6% and 68.1 ± 8.7% of baseline U_{Met}, respectively ($P < 0.001$; n = 6); whereas, Cl^- free conditions had no effect (101.3 ± 8.7% of baseline U_{Met}; $P > 0.1$; n = 6). Since some amino acid transporters are sensitive to certain inhibitors, L-Met uptake was also measured in presence of N-methylaminoisobutyric acid (MeIMB), 2-amino-2-norbornane-carboxylic acid (BCH) and N-ethylmaleimide (NEM) on the mucosal side. Results were compared to baseline using one-way ANOVA. Only NEM showed a decrease in U_{Met} to 44.6 ± 5.9% of baseline ($P < 0.05$; n = 7). Finally, U_{Met} was measured in Na^+-containing vs. Na^+-free conditions on the mucosal side at 10 different L-Met concentrations. The Na^+-dependent portion of U_{Met}, followed single-K_d Michaelis-Menten kinetics with K_m = 488 ± 170 µM ($P < 0.05$ to zero; n = 10) and U_{Met}-max = 32.1 ± 0.4 nmol·cm^{-2}·min^{-1} ($P < 0.001$ to zero; n = 10).

Conclusions: These results confirm that the transporter induced by a DL-Met containing diet is Na^+-dependent with apical localization. As the withdrawal of Cl^- had no significant effect on U_{Met}, primarily ASCT2 and B^0AT1 remain as candidates for this Na^+-dependent, chloride-independent part of U_{Met} (2). ASCT2 is known to be inhibited by NEM (3) and has a K_m of ~300 µM (4). Thus, it is likely that ASCT2 represents the transporter induced by a DL-Met-containing diet in the porcine jejunum.

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This study was supported by Evonik Nutrition & Care GmbH.

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59.

Characterization of the bovine TRPV3 channel in *Xenopus* oocytes

Charakterisierung des bovinen TRPV3 Kanals in Xenopus Oozyten

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Introduction: Emissions of nitrogenous compounds by dairy cattle play a considerable role in climate change. The major cause for the high nitrogen losses of cattle is related to the fermentational degradation of dietary protein into ammonia, which is absorbed via an electrogenic pathway across the ruminal epithelium, detoxified by the liver, and excreted by the kidney. From previous studies, bTRPV3 has emerged as a promising candidate mediating ruminal uptake of ammonium [1, 2]. The purpose of the present study was to analyze the single channel characteristics of bTRPV3 in an alternate expression system, to investigate the impact of expression on membrane permeability and cytosolic pH, and to establish antibodies for detection of the channel on the protein level.

Methods: The bTRPV3 channel was sequenced from ruminal tissue of cattle [3] and cloned into an expression vector. *Xenopus* oocytes were either injected with water (control) or strep-tagged cRNA encoding for bTRPV3 (30 ng). After an incubation period of at least four days, oocytes were studied using the patch clamp technique in the inside-out configuration (pipette: NH_4^+, bath: Na^+ or NH_4^+, all 96 mmol·l^{-1}). Measurements of membrane potential and pH_i were performed using double-barreled pH-sensitive microelectrodes. The cloned bTRPV3 sequence was used to screen for a suitable epitope for interaction with a commercial antibody.

Results: In single channel experiments in symmetrical NH_4^+, 90% patches from bTRPV3 cells showed single channel events versus 60% of patches from controls. The conductance was significantly higher in bTRPV3 patches ($p = 0.003$). When data were plotted in amplitude histograms, a peak around 40 pS emerged in both groups, which was judged to reflect expression of endogenous channels. In the bTRPV3 group only, a second peak emerged at > 100 pS with a mean conductance of 158 ± 12 pS (n = 11). When corrected for the lower concentration of oocyte buffer, this conductance was identical to that previously obtained for NH_4^+ in HEK-293 cells expressing bTRPV3 ($p = 0.786$). In Na^+ buffer, conductance to Na^+ was 101 ± 10 pS, again reflecting the concentration-corrected value in HEK-293 cells ($p = 0.838$). Endogenous channels were more frequently observed in control patches (57%; ~32 pS for Na^+; ~44 pS for NH_4^+) than in bTRPV3 patches (34%; ~36 pS for Na^+; ~48 pS for NH_4^+).

In oocytes studied with double-barreled pH-sensitive microelectrodes, the relative permeability to Na^+ or NH_4^+ versus $NMDG^+$ was significantly higher in bTRPV3 than in controls (both n = 17, $p < 0.02$). Exposure to 96 mmol·l-1 NH_4Cl for ten minutes led to a significant acidification ($p < 0.001$) in both bTRPV3 (pH_i = 6.33 ± 0.27) and control oocytes ($pH_i = 6.36 \pm 0.34$), but no differences emerged between the groups.

Specific binding of the antibody to the bTRPV3 epitope was verified in Western blots stained in parallel against the strep-tag of the bTRPV3 construct. Oocytes overexpressing bTRPV3, but not control oocytes, exhibited staining especially of the plasma membrane. In the ruminal epithelium, staining was located in the stratum spinosum and stratum granulosum.

Conclusion: Single channel and microelectrode experiments confirm that bTRPV3 conducts the NH_4^+ ion with a single channel conductance comparable to that previously observed in HEK-293 cells. In addition, channels with a much smaller conductance for Na^+ and NH_4^+ were observed both in overexpressing and control *Xenopus* oocytes. These endogenous channels did not cross-react with the commercial antibody used. Immunohistochemical staining of the bovine ruminal epithelium suggests that both the stratum spinosum and the stratum granulosum express bTRPV3 protein.

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Funding for this study was provided by the Deutsche Forschungsgemeinschaft (STU 258/7-1, F. Liebe) and by the „Sonnenfeld Stiftung“ (H. Liebe)

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60.

Adaptation mechanisms to hypoxia in glucose transport across lagomorph jejunum epithelium

Anpassungsmechanismen des intestinalen Glukosetransports über das lagomorphe Jejunumepithel an Hypoxie

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The gastrointestinal epithelium can cope with huge variations in blood flow and thus oxygenation. This means, the epithelial cells must have means to adapt to hypoxic conditions effectively. However, under several pathological conditions these adaptation mechanisms fail resulting in severe epithelial damage. A first step to prevent or ameliorate epithelial damage is to understand these mechanisms and their effects. Since sufficient energy supply is crucial especially under hypoxia and thus might be part of the adaptation, we investigated glucose transport mechanisms across jejunum epithelium under hypoxic conditions.

Methods: Lagomorph jejunal epithelia were incubated in Ussing chambers under short circuit conditions. Hypoxia was simulated by gassing with 99% N2 + 1% O2 (hypoxia) instead of 100% O2 (control). The activity of the sodium-coupled glucose transport via SGLT1 was assessed by measuring the increase of short circuit-current after addition of 2mM glucose to the mucosal buffer solution. We also measured transepithelial flux rates (Jms) of radioactively labelled glucose and ortho-methyl-glucose (OMG). Specific inhibitors for the glucose transport proteins SGLT1, glucose transporter (GLUT) 1 and GLUT2 were applied before measuring flux rates again. To investigate underlying mechanisms, epithelia were preincubated with antagonists and agonists of AMP-activated protein kinase (AMPK) and functional effects as well as changes in protein expression were assessed.

Results: We observed a decreased electrogenic response to mucosal addition of glucose after prolonged hypoxia compared to control conditions (one-way repeated measures ANOVA, $p<0.01$, $N = 7$). The response could be restored by preincubation with the AMPK-antagonist compound C (two-way repeated measures ANOVA, $p<0.05$, $N = 9$). Western Blot studies showed an increased phosphorylation of AMPK (pAMPK) under hypoxia as well as incubation with AMPK-agonists but a significant decrease in pAMPK under hypoxia after incubation with compound C (paired t-test, $p < 0.05$, $N = 6$). Under hypoxia, Jmsglucose and JmsOMG were not changed but rather showed a tendency to increase, while Jmsmannitol indicated no changes in paracellular conductance. STF-31, an inhibitor of GLUT1, significantly blocked Jmsglucose under hypoxic, but not under control conditions (paired t-test, $p<0.01$, $N = 6$).

Conclusions: The activity of SGLT1 is decreased under hypoxic conditions AMPK-dependently. However, transepithelial transport of glucose is kept up by recruitment of GLUT1 into the apical membrane under hypoxia.

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

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61.

Effect of rearing environment and diet on colon bacterial fermentation and barrier function in neonatal piglets

Einfluss einer der Umwelt und Diät auf die bakterielle Fermentation und Barrierefunktion im Kolon von neugeborenen Ferkeln

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Hyperprolifacy in sows has increased the use of artificial rearing systems with formula feeding for neonatal piglets. Recent data suggest that formula feeding and/or the neonatal environment lead to changes in jejunal physiology and lower lactose digestion capacity (1). Therefore, we determined whether the neonatal environment or diet affects jejunal lactase activity and how this influences the large intestinal luminal environment and epithelial reactions.

Methods: Forty-eight new-born piglets were randomly allocated into four different groups in a 2 x 2 arrangement with environment (sow-reared (Sow) or artificial rearing unit/isolator (Iso)) and diet (sow milk (SM) or formula (FO)) as main factors. Formula was based on skimmed milk powder and whey (22.6% CP, 20.0% EE, 46.0% lactose per kg DM). Sow milk for feeding of piglets kept in artificial rearing units (group Iso-SM) was obtained in the previous lactation from the same mothers. Piglets in Sow-FO groups were removed twice daily from their mother and fed formula, but effectively received both, sow milk and formula. At days 7 and 14 of life, n=6 piglets per group were euthanized for sampling of digesta and jejunum and colon tissue. Jejunum brush border membrane enzyme activity was performed as described previously (1). Colon microbial metabolites (D-/L-lactate, short chain fatty acids (SCFA), NH4, biogenic amines) were analysed as described (2). Cross sections from colon were stained with HE for crypt depth measurements and with Alcian blue pH 2.5-periodic acid Schiff procedure for counting goblet cells (GC) and to distinguish acidic, neutral and mixed acidic/neutral mucins. Expression of genes related to barrier function (*CLDN3, ZO-1, MUC2*), and immune reaction (*TNFα, IFNγ, IL-1β TLR2, TLR4*) was performed as described (1). Data were evaluated using GLM with environment and diet as main factors in SPSS.

Results: Both groups kept in isolators had lower daily gain and final body weight compared with sow-reared littermates ($P<0.05$). Both, rearing environment (isolator) and diet (formula) resulted in lower ($P<0.05$) activity of jejunal lactase at both time points, with lowest activities in Iso-FO group. This was associated with higher ($P<0.05$) lactose concentrations in jejunal and colon digesta. As a consequence, total D-/L-lactate, total and individual (mainly propionate, n-butyrate) SCFA, and biogenic amines were higher ($P<0.05$) in the colon of these piglets with highest values in Iso-FO group followed by Sow-FO. At day 7, crypt depth was lower with formula feeding, whereas the opposite was found on day 14 associated with cellular infiltrations. At day 7, mainly diet dependent differences were observed for total and neutral GC being higher in formula fed piglets, whereas more acidic GC where found in sow reared piglets ($P<0.05$). Similar patterns were observed after 14 days but environment had also an effect on acidic and neutral GC counts ($P<0.05$). Expression of *MUC2* and *TLR4* was lower in Sow-FO and Iso-FO piglets on both time points ($P<0.05$). Such diet-dependent effects were observed for the expression of *TNFα, IFNγ*, *CLDN3* and *ZO-1*.

Conclusions: We demonstrate that the neonatal environment and diet (formula vs. sow milk) can lead to reduced jejunal lactase activity, which is specifically amplified under high-lactose formula feeding. This leads to a massive increase in colon bacterial fermentation associated with an impaired barrier function. The cause for reduced jejunal lactase activity has yet not clearly been identified.

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This study was supported by the German Research Foundation (Pi 946/2-1).

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62.

Exploring the allergenic potential of the absorption enhancer caprate

Untersuchungen zum allergenen Potential des Absorptionsverstärkers Caprinsäure

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Outline: Absorption enhancers such as the medium chain fatty acid caprate are used to improve gastrointestinal uptake of pharmacologically active drugs. Caprate permeabilizes the epithelium by a translocation of tight junction proteins tricellulin and claudin-5 (1). The effects of caprate on the follicle-associated epithelium of Peyer‘s patches (PP) have not been analyzed yet. These results could illuminate the potential allergenic effects of caprate, as the Peyer‘s patch regulates antigen contact within the intestine.

Methods: Porcine PP and villous epithelium (VE) taken from the distal small intestine of adult individuals were mounted in conventional Ussing chambers and incubated with 0.5 or 1 mM caprate for 4 hours (n = 11 – 12). Transepithelial resistance was reported, and unidirectional paracellular marker flux was measured employing sodium fluorescein under voltage-clamp conditions (0 mV, n= 5 – 6). Data was expressed in mean and standard error of the mean. Statistical analysis was carried out employing Student“s t-test.

Results: Caprate did not affect transepithelial resistance in our current approach, as values remained stable for both tissues and both tested concentrations throughout the course of the experiment (4 h). After 4 hours of buffer incubation TER of VE dropped to 80.75% of initial values, compared to 89.02% ($p = 0.28$) and 88.53% ($p = 0.38$) for 0.5 and 1 mM of caprate, respectively. Values of PP tissue were 92.97 % for control conditions, compared to 88.56 % ($p = 0.59$) and 98.71 % ($p = 0.41$) for 0.5 and 1 mM caprate. However, in PP absolute values for transepithelial resistance were generally higher (1,75 fold, $^{**}p < 0{,}01$) and paracellular permeability for sodium fluorescein was lower compared to VE (0,14 fold, $^{***}p < 0{,}001$) reflecting the generally sealed paracellular pathway of PP (2).

Conclusions: In this experimental approach the absorption enhancer caprate did not significantly affect epithelial barrier function of villous epithelium and Peyer‘s patches. Transepithelial resistance of both tissues remained unchanged during incubation while in PP, the transepithelial resistance was higher and the paracellular permeability was lower compared to controls. This cannot be linked to caprate though, since a tighter epithelial barrier in PP has already been shown under physiologic conditions (2) and caprate has been shown to enhance paracellular permeability, instead (1). Thus, caprate does not significantly impair the tight epithelial barrier of the follicle-associated epithelium of PP and the allergenic potential appears to be minimal in our current model with our tested concentrations.

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This study was supported by a grant of the German Research Foundation, AM141/11-1.

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63.

Does butyrate have a protective effect on porcine colon epithelium under hypoxia?

Zeigt Butyrate einen protektiven Mechanismus am porcinen Colonepithel unter Hypoxie?

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Introduction: The short chain fatty acid butyrate is mainly produced by microbial fermentation in the porcine colon to large amounts. Thus, it is the key energy source for the epithelial cells and plays an important role for their homeostasis and growth. Former studies on rumen epithelium raised suspicion that butyrate may protect it from damage under hypoxia which is a common complication of various diseases like inflammation and cancer. The exact mechanisms have not been identified thoroughly yet, but an influence of butyrate as a histone deacetylase inhibitor and/or by effecting hypoxia inducible factor (HIF) or AMP-activated protein kinase (AMPK) are discussed. We hypothesized that butyrate has protective effects under hypoxia on porcine colon epithelium as well, because both epithelia are similar in function and transport mechanisms. We therefore tested the influence of butyrate on tissue integrity and gene expression in porcine colon epithelium under hypoxia *In vitro*.
Methods: Stripped colonic epithelia from fresh slaughtered pigs were mounted in Ussing chambers, which were gassed with 100% oxygen for 30min to equilibrate and incubated either in a buffer solution containing 50mM Na-butyrate („butyrate“) or 50mM NaCl („control“) instead. After equilibration, chambers of each incubation group were gassed differently: „normoxia“ with 100% oxygen and „hypoxia“ with 1% oxygen and 99% nitrogen. Electrophysiological parameters (short circuit current (I_{sc}) and tissue conductance (G_t)) were measured for the complete incubation time (2h). Subsequently, mRNA expression was measured using RT-qPCR for the following target genes which are involved in the transport of butyrate (-metabolites): Monocarboxylate Transporter (MCT)1, MCT2, Sodium-coupled Monocarboxylate Transporter (SMCT)1, Down-regulated In Adenoma (DRA) as well as Zonula occludens (ZO)1.
Results: Initial I_{sc} of the epithelia treated with butyrate tended to be lower compared to the control group. I_{sc} showed a significant decrease under hypoxia in both buffer solutions, but the relative decrease was significantly diminished by butyrate. ($N = 6$; Two Way Repeated Measurements ANOVA with subsequent Holm-Sidak-Comparison, $p < 0.05$). Hypoxia also caused an increase of Gt compared to normoxia. However, the hypoxia induced increase was significantly lower under butyrate incubation compared to the control group. ($N = 6$; Two Way Repeated Measurements ANOVA with subsequent Holm-Sidak-Comparison, $p < 0.05$). The RT-qPCR analyses of the gene expression showed no significant difference neither between incubation with or without butyrate nor between the different gassing conditions.
Conclusion: We found functional effects of hypoxia on porcine colon epithelium. The increased G_t illustrates a reduced viability and/or barrier function of the epithelium under hypoxia. This was ameliorated but not abolished by butyrate incubation. The active transport processes across the epithelium, shown by a decreased I_{sc}, were also influenced by hypoxia. Again, the alteration of active transport processes was ameliorated by butyrate incubation. Thus, butyrate obviously has protective short-term effects on porcine colon epithelium when exposed to hypoxia and thereby may prevents the porcine colon epithelium from damage. If the butyrate effect would be caused by HIF, gene expression alterations were visible. Nonetheless, the lack of significant differences could also be due the short incubation time in the Ussing chambers. Possibly the time frame was too short to modify gene transcription. However, this also indicates that another protective mechanism apart from gene expression is modulated by butyrate.

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64.

Effects of cholera toxin on the epithelial barrier in rat small intestine

Effekte von Choleratoxin auf die epitheliale Barriere im Rattendünndarm

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Cholera toxin is commonly known to induce chloride secretion in intestine. In recent years, the effects on epithelial barrier function have been discussed. Our current study has focused on analysis of cholera toxin on transepithelial resistance, and on tight junction proteins known as structural correlate of barrier function.
Methods: Specimens of rat ileum epithelial tissue preparations were mounted in Ussing chambers, transepithelial resistance was recorded, and cholera toxin was added on the mucosal side (1 µg/ml). Subsequently, expression and localization of claudins was analyzed and morphological studies were performed employing transmission electron microscopy.
Results: Cholera toxin induced a marked decrease of transepithelial resistance in rat ileum (34%, $n = 8$; $p < 0.01$, Student‘s t-test), whereas controls remained stable. Tissue protein preparations revealed an increase of claudin-2 signals in Western blots (174%, $n = 5$; $p < 0.05$, Student‘s t-test), and transmission electron microscopy showed a reduction in the number of microvilli after incubation with cholera toxin. Moreover, cholera toxin led to an increase in the intercellular space of enterocytes.
Conclusion: In accordance to the commonly known diarrhea-inducing effect of cholera toxin, our study revealed an effect on small intestinal barrier function and integrity, which might be also considered as a possible pathomechanism in discussions of prevention and therapeutic approaches. Moreover, as in colon an increase of tightening tight junction proteins has been reported (1), this indicates a segment-specific action of the enterotoxin in intestine.

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65.

A comparison of faecal and renal phosphorus excretion after excessive intake of monophosphate from calcium and potassium monophosphate in adult cats

Ein Vergleich der faecalen und renalen Phosphorausscheidung nach exzessiver Monophosphataufnahme aus Calcium- oder Kaliummonophosphat bei adulten Katzen

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Introduction: Complete and balanced cat foods consistently contain excessive phosphorus levels. Phosphates may originate from bones but inorganic phosphates are added in considerable amounts for technical reasons. Previous studies demonstrated adverse effects of high phosphorus intake on parameters of kidney function in healthy cats (1,2). In these studies, a large percentage of phosphorus was excreted by the kidneys. The hypothesis for the present study was that excess phosphorus originating from water soluble potassium monophosphate might be more available than phosphorus from calcium monophosphate which is not water soluble. This would then result in a higher percentage of the excess phosphorus being excreted by urine, which presents a potential risk for renal health.

Animals, materials and methods: Twenty-five adult healthy European shorthaired cats were fed two phosphorus excess diets (HP diets. Phosphorus intake see below). For diet HPCaP calcium monophosphate [Ca(H2PO4)2] was used as phosphorus source, whereas for diet HPKP potassium monophosphate [KH2PO4] was added. The Ca/P ratio was adjusted to 1.3/1 by calcium carbonate. Control diets (CONCaP and CONKP, respectively) contained only phosphorus from rice and meat in an amount that met the recommended daily allowance of phosphorus (3). All cats were fed the control diets for 29 days before they were either switched to the respective HP diet or remained on the corresponding control diet for 29 days. Faeces and urine were collected in the last 10 days of the trial. After a 14-day wash-out period the cats from the control group were switched to the HP diet and vice versa. Phosphorus in food, faeces and urine was measured by photometry after wet digestion.

Results: Phosphorus source did not affect phosphorus retention in HP diets. The way of phosphorus excretion, however, differed. The water soluble potassium monophosphate led to a higher renal phosphorus excretion than the water insoluble calcium monophosphate.

Group	n	Intake mg/kg BW	Faecal excretion mg/kg BW	Apparent digestibility %	Renal excretion mg/kg BW	Retention mg/kg BW
CONKP	13	73±6^{a}	34±7^{a}	54±9^{a}	15±4^{a}	25±8
HPKP	13	236±22^{b}	127±17^{b}	46±8^{b}	52±10^{b}	56±28^{b}
CONCaP	12	74±7^{a}	38±7^{a}	49±9^{b}	14±2^{a}	22±7^{a}
HPCaP	12	216±20^{c}	140±24^{b}	36±8^{c}	25±5^{c}	51±18^{b}

Means in the same column not sharing a superscript letter are significantly different (2way-ANOVA, Holm-Sidak; $p<0.05$)
BW=body weight

Conclusion: In cats the excessive intake of water soluble hydrogen phosphate from potassium phosphate leads to a higher renal phosphorus excretion than the excessive intake of hydrogen phosphate from calcium hydrogen phosphate. The latter is soluble in acid but not in water. This suggests a higher potential risk for renal damage by water soluble phosphorus sources.

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(3) NRC (2006): Nutrient requirements of dogs and cats.

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66.

Genetic selection on high daily egg mass production improved the utilization of dietary calcium for egg synthesis in laying hens

Genetische Selektion auf hohe tägliche Eimasseproduktion verbesserte die Kalziumverwertung aus dem Futter für die Eisynthese in Legehennen

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The calcium (Ca) requirement of laying hens is mainly determined by the quantity of Ca deposited in the calcified egg shell. Due to limited Ca mobilization from the medullary bone hens have to meet their Ca requirement through a sufficient intake of dietary Ca primarily. In this context, intensive genetic selection on high daily egg mass production (DEMP) significantly increased hens' daily Ca requirement and might have modified their efficiency in using dietary Ca further. Based on this hypothesis, the present study aimed to examine the utilization of dietary Ca by hens from high (up to 50 g DEMP/d) and low (up to 30 g DEMP/d) performing purebred layer lines and characterize potential line-dependent differences in hens' Ca metabolism.

Methods: Thirty-six laying hens from four genetically diverse purebred lines (WLA/R11: high/low performing white layers; BLA/L68: high/low performing brown layers; n=9 hens per line) were individually housed in metabolic cages with a commercial diet (43.8 g Ca/kg DM) and water for *ad libitum* consumption. In their 42nd week of age a balance trial was carried out for 5 days. At the beginning and the end of the trial hens' live body weight (BW) was recorded. Residual feed as well as weight and composition of laid eggs were recorded daily. Twice a day, excrements of each hen were collected entirely. Feed samples and freeze-dried excrements were analysed for dry matter and Ca concentration according to VDLUFA methods. Due to line-dependent differences in hens' live BW, balance parameters were related to hens' metabolic BW ($kg^{0.75}$). Total Ca retention was calculated by subtracting Ca excretion from Ca intake, whereas Ca retention in eggs was estimated by multiplying daily shell, yolk and albumen production with their average Ca concentration (370 mg/g shell, 1.4 mg/g yolk, 0.11 mg/g albumen). Subtracting Ca retention in eggs from total Ca retention remaining Ca retention in body was calculated further. The quotient of daily Ca retention in egg and daily Ca intake described the Ca utilization for egg synthesis. For statistical evaluation of data a one-factorial ANOVA with „line" as fixed effect was performed using SAS 9.4 procedure ANOVA (2012) with Student-Newman-Keuls test. Differences between lines were considered as significant for $p \leq 0.05$.

Results: During the entire experiment daily Ca intake and excretion did not differ between high and low performing hens with the exception for significantly lower Ca excretion by WLA hens ($p<0.05$; Table). According to their higher daily shell production (5.08±0.57 vs. 3.54±0.46 g/kg0.75/d; $p<0.01$), high performing hens absolutely retained more Ca in eggs than low performing ones ($p<0.05$). But, high as well as low performing hens deposited approximately 72% of retained Ca in eggs and did not differ in the remaining Ca retention in body. However, high performing hens, especially WLA, apparently utilized dietary Ca more efficient for egg synthesis than low performing hens ($p<0.01$).

Line	Ca Intake	Ca excretion	Total Ca retention	Egg Ca retention	Body Ca retention	Ca utilization for egg synthesis
	g/kg$^{0.75}$/d	g/kg$^{0.75}$/d	g/kg$^{0.75}$/d	g/kg$^{0.75}$/d	g/kg$^{0.75}$/d	%
WLA	4.70	1.82 ^{b}	2.88 ^{a}	1.89 ^{a}	0.99	34.7 ^{a}
BLA	4.79	2.39 ^{a}	2.40 ^{a}	1.90 ^{a}	0.50	27.0 ^{ab}
R11	4.01	2.45 ^{a}	1.56 ^{b}	1.16 ^{b}	0.40	21.2 ^{bc}
L68	4.16	2.29 ^{a}	1.87 ^{b}	1.30 ^{b}	0.60	23.3 ^{b}
PSEM	0.40	0.15	0.25	0.17	0.36	3.23
p-value	0.563	< 0.05	< 0.05	< 0.05	0.653	< 0.01

a-c: Values with different superscripts differ significantly within the same column ($p \leq 0.05$).

Conclusions: The present study indicates that a slight increase in dietary Ca consumption and especially a more efficient utilization of dietary Ca for egg synthesis ensure the higher retention of Ca in eggs of intensively selected, high performing hens. In order to understand the mode of action of such selection-induced metabolic adaptations the underlying molecular and cellular mechanisms should be examined in further studies in more detail.

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67.

Cluster analyses on the adaption of zinc reservoirs in weaned piglets challenged with short-term finely-graded reduction in dietary zinc supply

Clusteranalysen zur Anpassung der Zinkreservoirs in Absetzferkeln bei kurzfristiger, feinabgestufter Reduzierung der alimentären Zinkversorgung

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Data on the adaption of whole-body Zn homeostasis under the terms of short-term subclinical Zn deficiency is scarce. This study aimed in investigating the adaption of Zn pools in various biological matrices of weaned piglets by hierarchical average linkage cluster analyses.

Methods: 48 fully weaned piglets (50% male-castrated, 50% female, 8.5 ±0.27 kg initial weight) were assigned to one of 8 treatment groups according to life weight, litter and sex, receiving finely-graded differences in dietary Zn supply (28.1, 33.6, 38.8, 42.7, 47.5, 58.2, 67.8, 88.0 mg Zn/kg diet) by restrictive feeding (450 g/animal*day-1) as described previously (1). After precisely 8d, all animals were killed by bleeding under anesthesia without fasting. Total Zn concentrations were determined from feces, blood plasma, jejunum, colon, bone, liver, kidney, pancreas, heart muscle, skeletal muscle, mesenteric lymph nodes, thymus, spleen and epidermis by atomic absorption spectrophotometry (novAA 350, Analytik Jena AG). Data was analyzed using hierarchical average linkage cluster analyses by Euclidean distance (PROC CLUSTER, SAS 9.4, SAS Institute Inc.).

Results: Investigating the tissue Zn concentrations according to dietary treatment groups revealed two main clusters, separating the three highest Zn supplied groups (≥58.2 mg Zn/kg diet) from the five lowest supplied groups (<58.2 mg Zn/kg diet). Both main clusters were further separated into a total of four sub-clusters. These sub-clusters group animals in numerical order according to the dietary Zn concentrations they received during the 8d experimental period ((a) 28.1+33.6, (b) 38.8+42.7+47.5, (c) 58.2+67.8 and (d) 88.0 mg Zn/diet) (Figure 1). Clustering the tissue Zn concentrations between biological matrices separated feces Zn, femur Zn and blood plasma Zn from each other and the soft tissues, respectively. The soft tissues were further clustered into two main groups, separating liver, colon, jejunum and pancreas from all other soft tissues (data not shown).

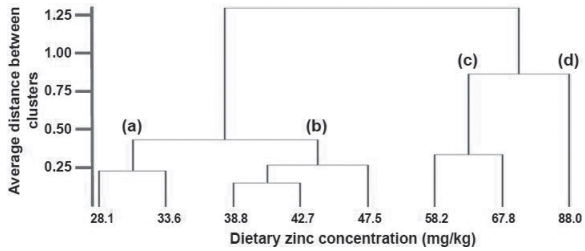

Figure 1 Average linkage cluster analysis between treatment groups

Conclusions: Based on earlier published data (1), using cluster analysis allowed a separation of sufficiently and deficiently Zn supplied groups (< or ≥58.2 mg Zn/kg diet, respectively) as well as a discrimination of groups that have been earlier shown (2) to express compensative stress responsive cellular programs (<38.0 mg Zn/kg diet). Clustering groups of biological matrices discriminated between excretion, storage and transport media (feces, femur, blood plasma) as well as soft tissues. The latter were further grouped in tissues potentially involved in Zn homeostatic regulation (jejunum, colon, liver, pancreas) and those which are affected by this regulation. This provides a first comparative view on the Zn homeostatic adaption to short-term insufficient dietary Zn supply.

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Supported by the Bayerische Arbeitsgemeinschaft Tierernährung (BAT) e.V..

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68.

Investigations on solubility of dietary phosphorus in water and acid medium from foods and mineral sources in companion and food producing animal nutrition

Untersuchungen zur Löslichkeit von Phosphor aus Futtermitteln und Mineralquellen in Wasser oder Säure in der Ernährung von Heimtieren und Lebensmittel produzierenden Tieren

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With regard to phosphorus (P), pet nutrition and food producing animal nutrition face different scenarios: in pets, P intake by far exceeds requirements; P originates not only from bones, meat and cereals, but also from large amounts of inorganic sources, which present a risk for chronic kidney disease (Dobenecker et al. 2017). By contrast, in food producing animals efforts are made to reduce inorganic P, and to increase P availability. P can only be absorbed, if it is soluble. Therefore information on the solubility of P is of interest in both scenarios. Testing In vitro solubility of P was also applied successfully to evaluate P binders for human patients with chronic kidney disease (Sheikh et al. 1989). In the present study we investigated P solubility in mineral compounds, ingredients, homemade diets and commercial pet food, and feed for swine and poultry.

Material and methods: Different mineral sources ($Ca(H_2PO_4)_2$, $CaHPO_4 \bullet 2H_2O$, $Ca(H_2PO_4)_2$, $CaHPO_4 \bullet 2H_2O$, $Ca_4Na(PO_4)_3$, KH_2PO_4, $K_4P_2O_7$, NaH_2PO_4, $Na_5P_3O_{10}$ (29 samples), ingredients, compound feeds and experimental diets were analyzed. Three g ground feed (fresh meat: 5 g) were soaked in water or hydrochloric acid (0.4 %) for 1 and 90 min. The samples were centrifuged and the supernatant was analyzed for P (photometric method after wet ashing, transforms all P into ortho-phosphate). For moist cat food there were data on Ca, Na, K and Cl. For these cat foods the Ca content was divided by 2 and subtracted from the P content. The result represents the P content, which cannot originate from bones (PNFB). To calculate potential compounds of P salts Cl was subtracted from the sum of Na and K. Linear regressions were calculated between water soluble P, Na+K-Cl and PNFB. For one data set on tripe-rice diets with various P supplements, data on postprandial (2 h) serum P concentration in dogs were available (Siedler et al. 2015).

Results: The solubility of the feed grade mineral P sources reflected the solubility of the purified chemical P salts, i.e. a solubility of >80 % in acid and/or in water. Variation coefficient of repeated sample assays was below 3 %. Cereals and plant proteins had a considerably lower solubility of P in water, than most animal protein sources. For acid solubility, the differences were less marked. P solubility in experimental diets reflected the P solubility of the P sources. Between pig and poultry feed and feed for different types of production there were no systematic differences. The percentage of acid soluble P increased with increasing P content, presumably because higher P contents originate from inorganic sources. Pet moist food showed high percentages of water soluble P. In moist cat food, there was a highly significant correlation between water soluble P and PNFB as well as Na+K-Cl ($p<0.001$). There was a correlation between the intake of water soluble P (after 1 min) and postprandial serum P levels in adult dogs ($R2=0.84$, $p<0.001$).

	n	Total P g/kg dry matter	Solubility % H_2O, 1 min	H_2O, 90 min	HCl, 1 min	HCl, 90 min
Moist dog food	13	15±3	47±6	44±12	47±6	62±11
Dry dog food	8	11±3	14±7	20±8	46±10	80±8
Moist cat food	25	16±0.7	33±13	35±13	49±11	72±11
Pig and poultry feed	64	7±2	31±11	48±13	51±7	63±8
Meat oven dried	2	11±0.08	50±7	67±14	39±6	61±0.3
Meat raw	3	9±2	69±3	73±2	59±3	69±10
Meat cooked	3	10±0.5	47±10	51±13	40±9	45±13
Wheat	9	3.7±0.4	15±5	42±8	40±8	50±3
Barley	8	3.7±0.5	32±11	65±8	41±8	47±3
Corn	6	3.11±0.3	60±13	81±11	51±11	59±6
Rape seed meal	4	12.3±0.8	9±1	13±1	45±2	59±18
Soy bean meal	5	6.8±0.2	42±23	57±5	16±3	21±3

Conclusion: The method is suitable to analyse P solubility in feed ingredients and compound feed. The postprandial serum P reflected P solubility of diets in dogs.

Siedler et al. 2015. 19. ESVCN Congress Toulouse, Sept. 17th-19th Proc. p. 128.
Dobenecker et al. 2017. J. Fel. Med. Surg. doi.org/10.1177/1098612X17710589

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69.

A simple indicator for phosphorus availability in growing pigs

Ein einfacher Indikator für die Verfügbarkeit des Phosphors bei wachsenden Schweinen

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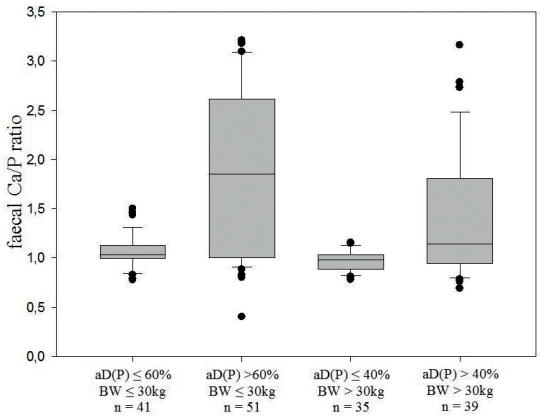
In a previous meta-analysis on calcium (Ca) and phosphorus (P) metabolism in different species(1), correlations between dietary intake and faecal excretion of Ca and P [modif. Lucas-tests(2)], respectively, reflected species-specific mechanisms of Ca and P metabolism. As a simplified method the comparison of Ca/P ratios in feed and faeces were used for demonstration of species differences(1). In the present meta-analysis, the same methods were applied to literature data on Ca and P digestibility in growing pigs fed diets with and without phytase, in order to identify diets with low P availablity.

Methods: Forty-six studies with data on intake and faecal excretion of P and Ca in trials with phytase supplemented diets (PHYT) and control diets (CON) in growing pigs were collected after a standard literature search. Linear regressions were calculated between intake and faecal excretion of Ca and of P for PHYT and CON diets. The mean true digestibility (tD) for Ca and P was calculated from the regression equations (tD (%) = [1-slope]*100). Regression lines of PHYT and CON diets were compared with the test of Ho. Data of trials with a dietary Ca/P ratio of ≥1.2/1 was grouped according to body weight (BW) (≤ 30kg or >30kg) and apparent digestibility (aD) of P (≤ 60% and ≤ 40% for pigs ≤ 30kg and >30kg BW respectively). Faecal Ca/P ratios of these groups were compared in a vertical box plot (Fig. 1; boxes represent the 25th and 75th percentile with median line, whiskers the 10th and 90th percentile and dots represent outliers).



Results & discussion: The PHYT group had a significantly higher tD of P than the CON group (69% vs. 56%, p<0.01). There was no significant difference between slopes of the regression lines of Ca intake vs. faecal Ca excretion between PHYT and CON (p=0.23), indicating no significant impact of phytase supplementation on tD of Ca (p=0.37). Independent of phytase supplementation, aD of Ca and P were significantly higher in pigs ≤ 30kg BW than in heavier pigs. The distribution of faecal Ca/P ratios was clearly different in groups with low and high aD of P (see Fig. 1). There were no data points of pigs ≤30 kg BW with an aD of P ≤60% and a faecal Ca/P ratio of >1.5/1. In pigs with a BW >30 kg, this cut-off occurred at a faecal Ca/P ratio of 1.2/1.

Conclusions: For common pig diets with a Ca/P ratio ≥1.2/1, faecal Ca/P ratio is indicative of the aD of P. This relative simple indicator can be used for example to evaluate the efficacy of phytase supplementation. On the other hand, faecal Ca/P ratios below the limits mentioned above are not directly conclusive of high or low aD of P, they can only hint at a potentially lower aD.

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70.

Correlations between different criteria of bone ash and phosphorus utilization in Japanese quail

Korrelationen zwischen verschiedenen Knochenasche-Daten und der Phosphorverwertung bei der Japanwachtel

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Bone ash criteria are often used as an indicator of the relative bioavailability of phosphorus (P) in poultry. Most studies use ash percentage, although it has been suggested that total ash amount is a more sensitive indicator in broilers (1). The most commonly used bone is the tibia. Another possible and less laborious indicator for bone mineralization is foot ash.

Japanese quails are important model animals for broiler chickens. Therefore this study investigated the suitability of foot or tibia ash as an indicator of P utilization (PU) using a very high number of Japanese quail. Additionally, the use of ash percentage was compared with the total ash amount.

Methods: A total of 887 unsexed Japanese quail were raised in their first 5 days of life in groups in floor pens and received a commercial starter diet. In order to let the birds express their full genetic potential of PU, a P deficient diet based on maize, maize starch, and soybean meal was fed from day 6 to 15. For measurements of PU and calcium utilization (CaU), quails were kept individually in metabolic cages from day 8 to 15. Following adaptation to the cages, feed intake voided excreta were quantified from day 10 to 15 and analysed for P and Ca (2). Quails were euthanized on day 15. The right *tibiotarsus* (tibia) and foot of each quail were dissected separately. Tibiae were cleaned from adhering tissues and cartilage caps. Feet were taken with skin, claws, and all tissues below the *articulatio intertarsalis*. Tibiae and feet were dried for 24 h at 103°C and ashed for 16 h at 550°C in a muffle furnace. Phenotypic correlations between measurements were estimated with a mixed linear model using ASReml.

Results: The mean value for PU was 71.4 %, and the range was from 21.5 to 87.4 %. Mean CaU was 60.6 % and ranged from 19.4 to 84.3 %. The amount of ash was 45.8 mg (19.2 - 83.5 mg) for tibia and 44.8 mg (19.6 - 83.6 mg) for foot. Ash in percentage of dry matter was 45.3 % (35.5 - 55.7 %) for tibia and 17.3 % (12.1 - 21.9 %) for foot. Estimated phenotypic correlations between these measurements are presented in the table. The correlations between PU or CaU and the ash amount were almost identical for tibia and foot. For both tibia and foot the bone ash amount provides higher correlations with PU and CaU than ash percentage.

	Tibia ash(% DM)	Foot ash (mg)	Foot ash(% DM)	PU(%)	CaU(%)
Tibia ash (mg)	0.567 (0.03)	0.901 (0.01)	0.510 (0.03)	0.511 (0.03)	0.646 (0.02)
Tibia ash (% DM)		0.536 (0.03)	0.688 (0.02)	0.348 (0.03)	0.561 (0.03)
Foot ash (mg)			0.590 (0.03)	0.527 (0.03)	0.662 (0.03)
Foot ash (% DM)				0.268 (0.04)	0.530 (0.03)
PU (%)					0.839 (0.01)

Phenotypic correlations are followed by their approximate standard errors

Conclusions: Total amount of bone ash is a better indicator of P utilization than ash concentration. Foot ash is as suitable as tibia ash, but easier to determine. In large populations, P utilization efficiency might be estimated from bone ash data. However, correlations do not appear close enough to estimate P utilization of individual birds.

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71.

Short-term kinetics of tissue zinc exchange in ^{65}Zn-labelled adult rats receiving sufficient dietary Zn supply

Zur kurzfristigen Kinetik des Gewebezinkaustauschs in 65Zn-markierten, adulten Ratten bei bedarfsdeckender Zinkversorgung

*Brugger D., Schlattl M., Buffler M., Windisch W. – Freising

The response of tissue zinc (Zn) exchange kinetics under the terms of basal physiological conditions (no Zn deficiency) is not well understood. Therefore, this study investigated the short-term adaption of tissue Zn in ^{65}Zn labelled adult rats receiving sufficient dietary Zn supply.

Methods: 32 adult female Sprague-Dawley rats (220 g/animal) were fed restrictively (8 g/animal*day-1) a Zn adequate (12 µg/g feed) semi-synthetic diet (34.8% corn starch, 28% sucrose, 20% acid extracted casein, 8% plant oil, 6% micronutrient premix, 3% cellulose, 0.2% DL-methionine) for 1 week. Subsequently, all animals were fed once the same basal diet but labeled with 0.5 kBq ^{65}Zn/µg of total dietary Zn (specific activity 523 Bq/µg). Each 4 animals were killed by decapitation under anesthesia at time points 0, 1, 2, 4, 8, 24, 28 and 32 h after complete consumption of the labeled feed (40 min after offering). Animals killed at 24, 28 and 32 h post-feeding of the 65Zn-labeled diet were offered an unlabeled daily feed portion to avoid catabolic processes. Rats were quantitatively dissected into Liver, GIT (gut + content), blood plasma, blood cake, muscle + fat, fur (skin + hair) and skeleton. Feces and urine were collected without losses. 65Zn-activity in tissues and excrement were analyzed by one-way ANOVA (time) and presented as percentage of the applied 65Zn dose.

Results: During the first 24h, nearly half of the alimentary ^{65}Zn has been absorbed. The residual ^{65}Zn was distributed to the GIT, feces, urine and fur. Within this period, 24% and 10% of the absorbed ^{65}Zn-activity accumulated in muscle + fat and the skeleton, respectively. Furthermore, a high incorporation of ^{65}Zn into liver (~9.9%) was recognized.

Time (h)	0	1	2	4	8	24	28	32
GIT	99.1^{a}	93.4^{ab}	87.4^{b}	77.2^{c}	59.8^{d}	42.1^{e}	33.7^{e}	33.8^{e}
Liver	0.3^{c}	1.8^{c}	4.2^{b}	7.7^{a}	10.8^{b}	9.3^{a}	8.7^{a}	7.6^{a}
Blood	0.1^{d}	0.3^{c}	0.6^{bc}	0.7^{b}	0.8^{ab}	0.9^{a}	0.8^{a}	0.8^{a}
Muscle + fat	0.3^{d}	1.6^{d}	3.4^{cd}	6.6^{c}	13.3^{b}	24.0^{a}	25.4^{a}	23.5^{a}
Skeleton	0.1^{d}	0.7^{d}	1.5^{d}	3.2^{c}	6.4^{b}	$^{9.9a}$	9.9^{a}	9.4^{a}
Remaining tissues	0.1^{d}	1.1^{c}	$3.6b^{c}$	4.5^{b}	6.7^{ab}	8.9^{a}	8.7^{a}	8.0^{a}
Feces	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	3.4^{b}	10.7^{a}	14.4^{a}
Urine	0.00^{b}	0.00^{b}	0.00^{b}	0.01^{b}	0.02^{b}	0.04^{a}	0.05^{a}	0.04^{a}

Means not sharing a common superscript differ by $P \leq 0.05$.

Conclusion: The observed relation in ^{65}Zn-incorporation into muscle + fat and bone corresponds to the long-term exchangeable Zn pools of both tissues. Furthermore, the response of ^{65}Zn-activity in liver over time suggests it represents a primary buffer for absorbed Zn, as it already reached a peak in ^{65}Zn activity after only 8 h post-feeding. Taken together, our data highlights the role of the skeleton as a quantitatively relevant and highly mobile Zn reservoir, which was also suggested in earlier studies (1, 2).

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Generously supported by the Deutsche Forschungsgemeinschaft (DFG, WI 1554/3 – 1).

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72.

Composition of different types of bones in weaned piglets supplied with graded dietary phosphorus levels

Zusammensetzung verschiedener Knochen bei Ferkeln in Abhängigkeit gestaffelter Phosphorgehalte im Futter

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The phosphorus (P) supply of pigs plays an important role in the mineralization of piglets‘ bones and is frequently discussed in cases of porcine skeleton disorders. Based on its finite global resources and adverse environmental impacts P should only be used sparingly in farm animal feed. However, it is still unclear to what extent moderate dietary P limitations might influence porcine bone health negatively [1]. For that reason, the aim of the present study was to examine variations in the mineralization of different types of bones in weaned piglets supplied with graded dietary P-levels and to give a recommendation concerning the most meaningful bone for assessment of P-supply.

Methods: Female and castrated male weaned German Landrace piglets (n=21; 28 days-old; mean BW: 8.7 ± 0.88 kg) were allocated to 3 groups (n=7) that were fed pelleted phytase-free diets with graded P-levels. Based on a total P-level of 6.7 g/kg DM (group M = medium), the level was reduced (group L = lower: 5.4 g P/kg DM) and increased (group H = higher: 8.0 g P/kg DM) by 20%, respectively. This experiment was performed in two trials. In trial 1 (T1) the dietary calcium level in all groups was 10.5 g/kg DM, whereas in trial 2 (T2) a constant dietary Ca:P-ratio was used (L: 7.1, M: 11.4, H: 15.3 g Ca /kg DM). Piglets were slaughtered at day 64 of life and femur (only the proximal part), humerus, metacarpale III and metacarpale IV bones (last ones in toto) were prepared and analysed. Proximate analysis was carried out according to VDLUFA methods after complete defattening (fat free: ff) of bones. Calcium and phosphorus were analysed after microwave ashing by atomic absorption spectrometry and colorimetry, respectively. Statistical evaluation was performed by one-way analysis of variance (ANOVA; fixed effect: dietary P-level) using SPSS Statistics (23.0).

Results: Crude ash levels (g/kg DM_{ff}) of femur, humerus, metacarpale III and IV (mean ± SD) of weaned piglets supplied with graded dietary phosphorus levels are represented in the following table. In both trials the low dietary P level (group L) led to significantly lower ash levels in all types of bones taken from the piglets. However, the high dietary P level (group H) did not increase the bone ash content in comparison to the medium dietary P level (group M).

	group	femur	humerus	metacarpale III	metacarpale IV
T1	**L**	445 ± 34.9 ^{A}	456 ± 28.6 ^{A}	464 ± 25.5 ^{A}	481 ± 45.0 ^{A}
T2		417 ± 52.6 ^{aA}	461 ± 25.0 ^{bA}	491 ± 33.4 ^{b}	471 ± 31.0 ^{b}
Ø T1 + T2		432 ± 44.8 ^{aA}	458 ± 26.1 ^{abA}	476 ± 31.5 ^{bA}	477 ± 38.1 ^{bA}
T1	**M**	532 ± 44.5 ^{B}	522 ± 17.2 ^{B}	525 ± 26.5 ^{B}	536 ± 32.2 ^{B}
T2		476 ± 51.4 ^{AB}	503 ± 14.1 ^{B}	512 ± 23.1	498 ± 35.5
Ø T1 + T2		502 ± 54.8 ^{B}	512 ± 17.8 ^{B}	518 ± 24.6 ^{B}	515 ± 38.0 ^{AB}
T1	**H**	542 ± 59.5 ^{B}	545 ± 11.7 ^{B}	541 ± 24.9 ^{B}	528 ± 26.2 ^{B}
T2		487 ± 53.2 ^{B}	501 ± 19.6 ^{B}	508 ± 16.2	477 ± 30.2
Ø T1 + T2		514 ± 61.3 ^{B}	523 ± 27.4 ^{B}	525 ± 26.4 ^{B}	503 ± 38.1 ^{B}

a, b indicate significant differences between type of bones within one trial (p<0.05); A, B indicate significant differences between feeding groups within one bone and trial (p<0.05)

Interestingly, the mineralization response of these examined bones with a low dietary P level (group L) was comparable between the first and the second trial of the experiment. This result indicated that a low dietary P supply affected the crude ash content of bones regardless of a constant dietary Ca-level (T1) or a constant Ca:P-ratio (T2). No significant differences could be found between humerus and metacarpale III and IV as well, whereas the phosphorus level of the femur (only the proximal part was analysed in contrast to other bones) showed lower ash levels.

Conclusions: The present study underlined the diagnostic benefit of bones for the assessment of P-supply in growing pigs. In contrast to published studies using parts of femur and humerus for P-diagnosis the present experiment emphasized the suitability of porcine metacarpale III and IV. Great advantages to analyse these bones are an easier dissection during the slaughtering process without destruction of valuable parts of pigs (economic aspects) and the usage of the whole bone (avoiding varying P-levels caused by analysis of various active areas of the bone).

[1] Rieger et al. (2017): Proc. Soc. Nutr. Physiol., p. 48

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73.

Case report: Evaluation of the zinc status in a horse

Fallbericht: Bewertung der Zinkversorgung eines Pferdes

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Testing blood/serum levels of trace elements in horses is very popular and offers a chance to evaluate the dietary supply of individuals independent of further circumstances like ingestion of counteracting compounds. But there are restrictions/disadvantages that are neglected frequently. The following case report demonstrates the possible course of action to interpret conspicuous blood values.

Methods: As the zinc values in serum of an adult horse (body weight: 625 kg; maintenance requirements) varied below reference values repeatedly (February – July 2017: 7.4 - 8.3 µmol/L; reference values: 9.2 – 19.9 µmol/L) without clinical symptoms, the institute was contacted regarding a feed advise for the horse. The daily ration of the horse included 12 kg hay and dry haylage, 1 kg straw, 0.26 alfalfa pellets, 0.16 kg beet pulp, 0.11 kg linseed, 0.04 kg linseed oil, 0.11 kg mineral supplement (per kg: 80 g Ca, 40 g P, 30 g Na, 8 g K, 20 g Mg, 5040 mg Zn, 15 mg Se, 1060 mg Cu, 17 mg I, 6000 mg Vit. E, 400,000 IU Vit. A and 40,000 IU Vit. D), 1 kg carrots and 0.5 kg apples. The daily nutrient supply of the horse was compared with official recommendations (1). For components with no declaration available, nutrient contents from literature were assumed (1). The hay and haylage were analysed before by an official laboratory regarding dry matter and mineral contents.

Results: The following table presents the comparison of recommended and actual amounts of nutrients in the daily ration of the mentioned horse.

Parameter	Recommended daily intake	Calculated amount in the daily ration
Dry matter (kg)	≥ 1.5 % of body weight --> 9.36 kg	11.2 kg (hay, haylage, straw)
Ca (g)	23	68.0
P (g)	14	34.7
Na (g)	24	11.0
Zn (mg)	500	992
Cu (mg)	125	175

The only parameter which had to be adjusted was the Na content, thus the additional offer of a salt-lick-stone was recommended. The relatively high amounts of Ca, P and Zn in the ration were achieved mainly because of the concentrations in the hay/haylage (50.4 g Ca, 27.6 g P and 396 mg Zn in the daily ration) and in the mineral feed (8.8 g Ca, 4.4 g P and 554 mg Zn in the daily ration). Because of the calculations“ results a hair analysis was performed subsequently. The zinc concentration in the hair amounted to 168 mg Zn/kg dry matter.

Conclusion: The calculation revealed no hint on a primary zinc deficiency, as long as the whole ration (especially the mineral feed) was ingested completely. A secondary zinc deficiency cannot be ruled out (e.g.: no information was given about the iron content, which can vary widely), but it is unlikely because of the total amount of Zn. In the preliminary report there was no hint given on stressors (like chronic diseases or inflammation), which could cause a decrease in serum Zn content (2).

According to Ratjen et al. (2017; Ref. 3) hair of healthy German Riding Horses contains 125-244 mg Zn/ kg dry matter (mane hair) and 115-179 mg Zn/ kg dry matter (top hair). This quite insensitive but longterm parameter supported the assumption of an adequate amount of zinc in the ration. This case demonstrates the difficulty of a statement regarding zinc supply in horses via blood samples, as it was reported in other studies before (4).

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3) RATJEN, A., M. ANKE and M. FÜRLL (2017), Pferdeheilkunde 33, 1, 59-65

4) KREYENBERG, K. (2003): Zinkserumresponse beim Pferd nach oraler Verabreichung von unterschiedlichen Zinkverbindungen [Equine zinc serum response after oral supplementation of different zinc compounds], Thesis, München

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74.

Influence of different iron supplementation strategies on oxidative stress parameters in the liver tissue of suckling piglets

Einfluss verschiedener Eisensupplementierungsstrategien auf Kenngrößen des oxidativen Stresses in Lebergewebe von Saugferkeln

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Iron deficiency anemia represents the most common deficiency disease in piglet production. High performances result in an increased iron demand during the first days of life, which cannot be met by the native iron content in sow"s milk. Therefore parenteral iron supplementation is a common practice to avoid iron deficiency anemia in piglets bypassing the physiological homeostasis. Due to the toxic potential of free iron, symptoms of oxidative stress including edema, tissue necrosis and DNA damage could be observed (1). In order to avoid these negative effects of oxidative stress due to iron overload, this study aimed into investigation of different oral iron application regimes as alternative supplementation strategies.

Methods: 20 sows (DL) were divided in two groups and fed with isoenergetic, isonitrogenic diets differing in iron content („-Fe": 114 ppm vs „+Fe": 256 ppm) from insemination to farrowing. 6 piglets from 17 litters (7 „-Fe" and 10 „+Fe") each were treated with different iron supplementation strategies. Each 1 piglet was supplemented with 200mg iron(II)dextran via intramuscular injection at day 5 p.p. serving as positive control. Each 2 piglets received iron orally as paste (100mg iron(II)fumarate per dose) at day 1 p.p. and day 5 p.p., respectively. Repeated oral iron supplementation (2 times 100mg iron(II)fumarate) was administered to two piglets at day 1 p.p. and 14 p.p. or day 5 p.p. and day 14 p.p. One piglet from each litter served as negative control (no iron supplementation). All Piglets were euthanized at day 21 p.p. and glutathione and α-Tocopherol contents in liver tissue were analyzed with HPLC. Liver iron contents were determined by AAS. Statistical analysis was done by multifactorial ANOVA.

Results: No differences of oxidative stress parameters in piglet's liver tissue could be shown due to maternal iron supply during pregnancy. Reduced form of glutathione (GSH) increased significantly in parenteral iron supplemented positive controls compared to other treatments. Repeated oral iron application at day 5 + 14 showed numerically higher GSH contents in contrast to negative control and the other oral supplementation strategies. Content of the oxidized form of glutathione (GSSG) was two and three fold higher and ratio GSH:GSSG lower in oral groups day 1 day 1 + 14, respectively compared to control groups. α-Tocopherol contents in liver tissue didn"t differ between any treatment groups. Liver iron content was significantly higher in positive control.

	oral Fe				**control**			
	day 1	**day 1+14**	**day 5**	**day 5+14**	**positive**	**negative**	**SEM**	**p-value**
GSH (µmol/g DM)	11.7^{bc}	10.5^{c}	11.8^{bc}	15.3^{b}	19.0^{a}	13.0^{bc}	1.1	<.0001
GSSG (nmol/g DM)	42.5^{ab}	65.2^{a}	20.2^{b}	16.1^{b}	25.6^{b}	15.4^{b}	9.4	0.004
ratio GSH:GSSG	464.9^{cd}	391.6^{d}	740.9^{bcd}	1388.2^{a}	861.9^{ab}	1083.7^{ab}	114.0	<.0001
α-Tocopherol (nmol/g DM)	113.3	109.5	114.7	103.6	88.9	101.0	11.2	0.543
liver iron, mg/kg DM	124.9^{b}	190.3^{b}	158.1^{b}	225.0^{b}	833.9^{a}	98.6^{b}	52.8	0.0001

Conclusion: In contrast to oral supplementation strategies, parenteral iron supplementation, bypassing physiological homeostasis, induces activation of the antioxidative glutathione system (higher hepatic GSH contents). However cellular redox status still seems to stay balanced (no excessive shift towards ROS) compared to negative control. Interestingly, oral iron application at day 1 p.p. either onetime or repeated, seems to increase oxidative stress in liver tissue level, which cannot be explained by correlation to iron status (neither iron overload nor iron deficiency in comparison to control groups). These findings need further investigations.

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This study was supported by the Bayerische Arbeitsgemeinschaft für Tierernährung (BAT e.V.)

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75.

Effects of algae β-glucan supplementation on the performance and the intestinal immune system of weaned piglets

Effekte von Algen-β-Glukanen auf die Leistung und das intestinale Immunsystem von Absetzferkeln

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Oral administration of β-glucans from yeast and fungi has been shown to influence the immune response, and it has been suggested that β-glucans may be able to prime the immune system to increase resistance against invading pathogens (1). However, studies investigating the effect of algae β-glucans are rare. We hypothesized that dietary supplementation of β-glucans from the algae *Euglena gracilis* stimulates the intestinal immune system of early-weaned piglets.

Methods: 66 male weaned piglets ((DE x DL) x Pietrain), aged 21 days with 6.68 ± 0.99 kg body weight (mean ± SD), were allocated to 22 boxes with each 3 piglets in 2 successive experimental runs and were phase-fed 2 different diets during days 1-16 and 17-35. One group of 24 piglets received corn-wheat-soybean meal based basal diets only (control group). Two further groups of 21 piglets each received the same basal diets supplemented with the β-glucan preparation AlgamuneTM AM (53% ß-glucans as analyzed; >90% beta-1,3 linkages; Algal Scientific, USA) at 100 or 200 mg/kg (groups A100 and A200), corresponding to 53 and 106 mg ß-glucans/ kg diet in groups A100 and A200, respectively. Body weights and feed intake were recorded. After 35 days, segments of duodenum, proximal, and mid jejunum were collected, rinsed with 0.9% NaCl solution and stored into 10% buffered formalin prior to embedding in paraffin and Giemsa staining for examination of gut morphology (2) and for inflammatory scoring based on Day et al. (3). Mucosa of mid jejunum and proximal colon was collected for RNA isolation, cDNA synthesis and RT-PCR of pro-inflammatory, nutrient transporter, and tight-junction (TJ) genes (2). Feces was collected from the rectum to determine apparent total tract nutrient digestibility (2). The data were analysed by ANOVA with fixed factors treatment, experimental run, and their interaction, but the histopathological scoring data set was analysed with the non-parametric Kruskal Wallis test.

Results: After 35 days, feed intake (22.8±2.2, 24.3±2.3 and 24.8±2.3 kg in Control, A100 and A200 groups), weight gains (16.4±1.26, 17.4±1.65 and 17.8±1.43 kg), and the feed:gain ratio (1.39±0.04, 1.40±0.07 and 1.39±0.06) were similar in all experimental groups (P>0.10). Concomitantly, the digestibility of DM, OM, CA, CP, CF, and lipids, the gene expression of the monosaccharide transporters GLUT2, GLUT5, SGLT1 and of the peptide transporter PEPT1 in the intestinal mucosa, and the gut morphology were not different among groups (P>0.05). Supplementation of Algamune did not affect the integrity of the gut mucosa as evaluated by scoring of mucosal fibrosis and villus epithelial injury (P>0.10). Likewise, the gene expression of the TJ proteins CLDN3, OCLN and ZO-1 were not affected by Algamune supplementation (P>0.05). Algae β-glucan supplementation did not influence the intestinal immune system in the healthy piglets as evaluated by counts of intraepithelial lymphocytes, goblet cells, and of lymphocytes, eosinophils and neutrophils in the lamina propria (LP), with two exceptions: in the mid jejunum, no. of lymphocytes in the LP was higher by 19% in group A200 (P<0.001) and no. of goblet cells was higher by 45% (A100) and 60% (A200) compared to the Control group (P=0.06). The mucosal expression of the pro-inflammatory cytokines IL1B, IL8 and TNF was similar in all groups (P>0.10), but that of the intercellular adhesion molecule ICAM1 was higher in the colon of the Algamune supplemented groups compared to the control (P=0.022).

Conclusion: The supplementation of algae β-glucans neither affected piglet performance nor the integrity of the gut mucosa. The abundance of intestinal immune cells and cytokine expression were slightly affected. These results indicate that *E. gracilis* β-glucan supplementation in early-weaned healthy piglets at the tested dosages is not helpful to stimulate the intestinal immune system.

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76.

Effect of high or low dietary zinc oxide or Zn-Lysinate on the colon microbiome in weaned piglets

Einfluss hoher oder niedriger Diätkonzentrationen von Zinkoxid oder Zn-Lysinat auf das Mikrobiom im Kolon von Absetzferkeln

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The influence of high dietary levels of zinc oxide (ZnO) on the intestinal microbiota and their activity along the pig gastrointestinal tract has been studied intensively during the past years (1). In addition, high levels of dietary ZnO have been associated with an increased abundance of certain antibiotic resistance genes and multi-resistant *Escherichia coli* strains (2). So far, little is it yet known about the effect of organic zinc sources. Thus, we compared the influence of high or low dietary ZnO with Zn-Lysinate on the intestinal microbial metagenome, metabolic activity, and occurrence of antibiotic resistance genes in the colon of weaned piglets.
Methods: A total of n=40 weaning piglets randomly assigned to one of four groups. Dietary zinc level was adjusted to either 40 or 110 ppm with ZnO (110 ZnO) or 110 ppm Zn-Lysinate (adjusted for total Lysine; 110 Zn-Lys), or to 2500 ppm with ZnO (2500 ZnO). After three weeks, piglets were euthanized and digesta samples taken for analysis. Zinc fractions (i.e. free zinc, protein-bound zinc, total zinc) and bacterial metabolites (D-/L-lactate, short chain fatty acids (SCFA), ammonia) were analysed as described (1). Metagenomic sequencing of colon microbiota was performed with DNA extracts using Illumina NextSeq500 high output. Bacterial taxa at species level were identified from quality-checked metagenomic reads using the SLIMM tool (3). Relative species abundances were used for further analysis of ecological indices and identification of unique and common taxa between groups. Assignment of metagenomic reads into Gene Ontologies (GO) was done using the EBI metagenomics database. Differential abundance of bacterial taxa and functional genes was analyzed using partial least squares discriminant analysis (PLS-DA) and VIP scoring in R. Metagenomic reads were also checked for presence of antibiotic (AR) and multidrug resistance (MDR) genes.
Results: Growth performance and health status did not differ significantly. Highest ($P<0.05$) concentrations of total zinc, free zinc and protein-bound zinc were determined in group 2500 ZnO, but did not differ between the other groups. Lowest concentration of total and individual SCFA as well as ammonia was determined in 2500 ZnO group as compared with 40 ZnO, 110 ZnO and 110 ZnLys ($P<0.05$), whereas highest concentration of total SCFA, propionate and n-butyrate was found in the 110 ZnLys group ($P<0.05$). The number of bacterial taxa was lowest with ZnOLys, whereas lowest diversity was observed with 2500ZnO ($P<0.05$). At genus level, no differences between 40 ZnO, 110 ZnO and 110 ZnLys were observed, whereas 2500 ZnO showed a strong influence on many genera (e.g. lower abundance of *Megasphaera, Dialister, Ruminococcus*, higher abundance of *Bacteroides, Faecalibacterium, Blautia, Coprococcus*). However, at species level, a clear grouping according to dietary zinc concentration and source was observed, indicating different effects for ZnO or Zn-lysinate as zinc source. Among several unclassified *Prevotella, Bacteroides* and *Clostridium spp*. that differed between the groups, a propionate producing *Dialister succinatiphilus* was highly abundant in the 110 ZnLys group, which may be linked to the highest propionate values in this group. GO analysis revealed significant differences in metabolic function related to sporulation, stress response, mineral and carbohydrate metabolism. Finally, the abundance of AR genes showed a high variability but the cumulative abundance did not differ between groups. However, a higher abundance of MDR genes was observed in the 2500 ZnO group ($P<0.05$), suggesting positive selection by high luminal zinc concentration.
Conclusions: The study confirms the strong influence of very high dietary zinc concentrations on the large intestinal microbiome in weaned piglets, and also reveals that chemical form at lower zinc concentrations can change microbial composition at species level, coinciding with altered function and activity. Whether these effects are finally beneficial for the host needs further elucidation.

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77.

Effects of *Scrophularia striata extract* supplementation on the rumen microbiome and fermentation *In vitro*

Effekte von Scrophularia striata Extrakt auf die Pansenmikroben und Fermentation In vitro

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To promote efficient ruminal fermentation, diets high in energy and protein are commonly fed. This often results in ruminal dybiosis and long term health and productivity problems for the animal. The addition of feed additives such as ionophores was previously used to reduce the negative impacts of high energy diets, but has been banned in the European Union. Plant extract from the Iranian herb *Scrophularia striata* has been previously shown to have positive effects on *In vitro* rumen fermentation efficiency but the impacts on rumen microbiota are unknown. This study investigated the effects of different *S. striata* doses on *In vitro* rumen microbiome and the associated fermentation, in comparison to ionophore supplementation.

Methods: Using an *In vitro* rumen simulation system (Rusitec), the supplementation of a low (Scro_Low; 40 mg/g DM), and high (Scro_High; 80 mg/g DM) doses of *S. striata* in a 50:50 forage to concentrate diet was compared to an ionophore supplement (Monensin; 0.83 mg/g DM) and an unsupplemented Control treatment. Rumen fermentation was assessed using volatile fatty acids (VFA) using gas chromotography (GC, Model 8060 MS DPFC, No.:950713, Fisons, Rodena, Italy). Ammonia was determining by the colorimetric indophenols method with a light absorbance measured at 655nm, using a photometer (UV-1800 Shimadzu Corporation, Kyoto, Japan). Rusitec pH (pH meter; seven MultiTM, Mettler-Toledo GmbH, Schwerzenbach, Switzerland), and methane (infrared detection; ATEX biogas Monitor Check BM 2000, Ansyco, Karlsruhe, Germany) were also analyzed as markers for rumen fermentation. To identify alterations in the microbial community, samples were both sequenced for 16S rRNA bacteria and analyzed for archaea, fungi and protozoa using quantitative PCR. Samples were analyzed for variance with the Proc Mixed procedure of SAS with treatment as the fixed effect, and randomization by run.

Results: Rusitec fermenter pH was not effected by the treatments. The supplementation of additives resulted in decreased CH4 and NH3, with Scro_High having the least NH3 ($P < 0.001$) and Monensin having the least CH4 production. The addition of ionophore suppressed VFA production compared to supplementation with *S. striata* which increased VFA *In vitro* ($P = 0.04$). Supplementation of additives decreased the diversity of the rumen microbiome ($P < 0.001$) without impacting overall abundance ($P = 0.10$). Quantitative PCR showed a complete removal of protozoa under Monensin supplementation but an increase in protozoa in the Scro_High treatment ($P = 0.05$). Total copy numbers of archaea were reduced in both the Monensin and Scro_High treatments but significantly increased with Scro_Low supplementation ($P < 0.001$). Statistical analysis of the sequencing data showed significant effects of treatments on all phyla ($P < 0.05$). At the genera level, 43 of 62 identified genera were significantly affected by treatment. Supplementation with Monensin increased *Bacteroides*, *Butryricimonas*, *Parabacteroides*, *Fusobacterium*, *Succinivibrio* and a number of uncultured gut-associated microbes ($P < 0.05$). Supplementation with *S. striata* increased the relative abundance of *Paludibacter*, *Fibrobacter*, *Pseudobutyrivibrio*, *Roseburia*, *Sphaerochaeta*, and *Treponema* ($P < 0.05$). Correlation of significant genera with rumen fermentation parameters showed a suppression of correlated methane and ammonia producers with supplementation of all additives. All genera which increased significantly with Monensin showed negative correlations ($-0.76 < r < -0.56$; $P \leq 0.004$) with CH4 and some with VFA production ($-0.73 < r < -0.61$; $P \leq 0.001$). *Fibrobacter* and *Paludibacter*, while having low relative abundance in this study, showed a strong positive correlation (($0.78 < r < -0.85$; $P < 0.0001$) with VFA production, and were promoted with the addition of *S. striata.*

Conclusions: The addition of *S. striata* clearly showed modulation of rumen fermentation towards increased efficiency while still maintaining a moderate microbial diversity. Thus, the use of such plant extracts to promote microbial stability through diversity is promising and these effects need to be verified *In vivo*.

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78.

Circadian eating behaviour of sheep fed diets supplemented with menthol-based bioactive lipid compounds

Zirkadianes Fressverhalten von Schafen bei Supplementierung Menthol-basierter bioaktiver Lipidzusätze

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Several plant bioactive compounds are increasingly evaluated for use as feed additives to improve production performance, health status and welfare of farm animals while offering environmental advantages. They may also affect eating behaviour of animals depending upon the compounds present in feeds. Menthol is one of the plant bioactive lipid compounds (PBLC) showing many beneficial biological activities. Dietary PBLC could potentially alter eating pattern of animals due to their distinctive odour, taste and olfactory modulation effect. Therefore, an experiment was conducted to study the effects of dietary PBLC with menthol as a lead substance on eating behaviour of sheep.

Methods: Twenty four growing Suffolk sheep at two experimental runs (each run with 12 sheep) were equally divided into three dietary treatments, i.e., control (diet 1; without PBLC), low dose (80 mg/d) of PBLC (OAX17, PerformaNat GmbH, Germany; diet 2) and high dose (160 mg/d) of PBLC (diet 3) in a randomised block design based on initial body weight and sex. Sheep in all groups were fed meadow hay ad libitum plus 600 g/d pelleted concentrate diets to meet their nutrient requirements. All diets had similar feed ingredient and chemical composition except PBLC. The daily dose of PBLC was supplied with the concentrate that was provided in three equal portions á 200 g at 07:00, 11:00 and 15:00 h. Feed intake and eating pattern data were recorded after 1 wk of adaptation for 3 wk using an automatic transponder-operated feeding system with pneumatically driven locking gates. During the last week, data of eating time and frequency (opening and closing of the locking gates) were determined with hourly resolution for each animal to determine the circadian eating profile. Data were analysed using mixed model procedures of SAS.

Results: Hay intake (as-fed) tended to be greater for the PBLC groups (average of 3 wk = 4.98 vs. 5.51 kg/wk; P=0.087), and thus total fed intake tended to increase (P=0.087) by 5.64% for the PBLC groups. Eating time tended to increase (264, 263 and 290 min/d; P=0.080) for the high concentrations of PBLC, but was not affected by wk (P=0.17) and diet × wk interaction (P=0.18). Eating frequency tended to increase with greater levels of PBLC (P=0.053), and tended to decrease over time from wk 2 to wk 4 (277, 270 and 269 gate openings/d, respectively; P=0.078), but was not affected by diet × wk interaction (P=0.34).

In wk 4, hourly eating time (min/h) showed circadian variation (P<0.001), but was not affected by the diet (P=0.17) and the interaction between diet × hour of day (P=0.32). However, total eating time during concentrate feeding hours tended to increase linearly with greater concentrations of PBLC (95.8, 98.0 and 104 min/3 h; P=0.082). Eating time during other hours (159, 169 and 182 min/21 h; P=0.12) was not affected by the diets due to a high coefficient of variation (16.3%); nonetheless, numerically higher values were observed for diet 3. Eating frequency was affected by the diet × hour interaction (P<0.001) during the 24 h clock period. For diet 2, eating frequency was greater at 08:00 h (P<0.001), 15:00 h (P<0.001), 16:00 h (P=0.0013) and 19:00 h (P=0.006) compared with diet 1. For diet 3, eating frequency was greater at 08:00 h (P=0.032), 16:00 h (P=0.0013) and 19:00 h (P=0.031) compared with diet 1. Overall, eating frequency during concentrate offering hours followed a quadratic response (P=0.082) (89, 106 and 89 times for diet 1, 2, and 3, respectively). The total eating frequency in other hours tended to increase linearly (P=0.089) with increasing concentration of PBLC in the diets (137, 165 and 169 times for diet 1, 2 and 3, respectively).

Conclusions: Circadian eating pattern of animals may be altered by diets containing PBLC, which is not only attributed to the presence of compounds in diets during feeding hours, but persists during post feeding hours. This altered behaviour may improve hay intake in sheep. In future studies, it would be interesting to investigate if the circadian change in feeding pattern may relate to circadian clock gene expression due to PBLC.

This study was conducted with partial funding by PerformaNat GmbH, Germany. AKP acknowledges the Fellowship of the Alexander von Humboldt Foundation.

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79.

Case report: Impaired locomotion in fattening turkeys due to vitamin A deficiency?

Fallbericht: Auftreten gestörter Bewegungsabläufe bei jungen Mastputen infolge eines Vitamin A-Mangels?

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Abnormal gait in turkey flocks is an obvious sign of a disease, causing a reduction in mobility, that maybe end in losses of animals. However, this is a rather uncommon clinical sign in turkeys [1]. The aetiology of gait problems in poultry is diverse and complex. Keeping in mind the role of diet composition, feeding trials are sometimes the last chance to clarify the reason for reported health problems (missing signs of an infection). The question in this diagnostic study was whether a distinct diet from the field could be the cause of abnormal gait of turkeys seen in large production units.

Methods: A total of 48 ♀ turkey poults (BUT-Big 6, 1-d old) were divided into two groups. The first group (control) was fed starter (first 4 weeks) and grower diets A (until trials end at end of week 8) whereas the second group received the conspicuous diets B in the starter and the grower period. The four diets were obtained from two different farms, without and with the reported abnormal gait. Diets were identical in all feed materials (wheat, corn, soybean meal, rapeseed meal, minerals, etc.) and labeled energy and nutrient contents. Body weight was recorded weekly. The dissections were performed in bi-weekly intervals. Serum and liver samples were collected at dissection. Parts of CNS and organs (oesophagus, proventriculus) were examined histologically. Statistical analyses were done by a pair-wise comparison for independent samples using the t-test, in the case of non-normally distributed data, differences in parameters between groups were assessed by using the Wilcoxon signed-rank test. All statements of statistical significance are based upon p-values smaller than 0.05.

Results: Both diets (A and B) had an almost identical chemical composition and energy content at each fattening phase (A: 12.4/13.4; B: 12.6/13.2 MJ AME_N/kg DM). However, the content of vitamin A was the main difference between the four diets. The levels of vitamin A in the starter and grower diets of the control group were 7168 and 5213 IU/kg diet, but <1000 IU/kg diet from the affected flocks. An incoordination in gait was the earliest sign in the experimental group (6th week of life). However, during the whole study there were no marked losses in both groups (only one in experimental group). Birds in the experimental group had a higher water:feed intake ratio compared to control one (2.86 vs. 2.58). Birds in control group had numerical higher final body weight (3839 g) than experimental one (3712 g). After only two weeks of feeding, vitamin A content in the liver was significantly decreased in the experimental group (1.81 mg/kg vs. 17.9 mg/kg). The contents of vitamin A in serum of birds in control group were almost constant during the whole trial (1.1 mg/L). Already after two weeks the level of vitamin A in serum was only 0.2 mg/L in experimental group. The level of uric acid in serum of birds in the experimental group increased gradually with age of birds (from 9.91 at 2nd week to 12.8 mg/dL at 8th week) vs. 3.38 mg/dL at 8th week for control one. Only birds in the experimental group had squamous metaplasia in the oesophagus, proventriculus and bursa of Fabricius. No evidence of further histopathological alterations was seen in CNS, eyes, sciatic nerve and pectoral muscle for both groups.

Table 1: Impacts of diets on total feed and water intakes, growth rate, contents of vitamin A in the liver and uric acid in serum of young turkeys fed different dietary vitamin A levels

Group	Feed intake/ bird[g]	Water intake/ bird[g]	Body weight-final[g]	Vit. A in liver[mg/kg]	Vit. A in serum[mg/L]	Uric acid in serum[mg/dL]
Control	6797	17539	3839±430	29.5^{a}±7.2	1.11^{a}±0.12	3.38^{b}±0.84
Experimental	6664	19119	3712±44	0.09^{b}±0.1	0.22^{b}±0.05	12.8^{a}±3.21

$^{a, b}$ means in the same column differ significantly ($p < 0.05$)

Conclusion: The striking gait seems to be caused by a severe continuous dietary vitamin A deficiency. The levels of vitamin A in liver and serum were affected significantly by dietary supply. The concentration of uric acid in serum was influenced by the content of vitamin A in the blood. Hyperuricemia could be a key risk factor for abnormal gait but the mechanism is still unclear.

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80.

Effects of a probiotic on B-cells from German Landrace sows

Effekte eines Probiotikums auf B-Zellen von Schweinen der Deutschen Landrasse

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Enterococcus faecium (*E. faecium*), a gram positive lactic acid-producing bacterium is a licensed probiotic for pigs and piglets. Feeding *E. faecium* can promote growth performance and health of weaned piglets. However, the underlying mechanisms of action of probiotic additives are still unknown as they may directly influence immune cells or change the intestinal milieu. In the light of the reduction of antibiotics, this study aims to enlighten the mechanisms of probiotics, in particular the direct effect of *E. faecium* on B-cells as a major component of the adaptive immune response.

Methods: Cell culture experiments were conducted with primary cultured porcine immune cells of German Landrace pigs (N = 6) in a co-culture with either vital or inactivated *E. faecium*. For validation, immune cells from blood were repeatedly taken from the same animals. 1 x 10^6 peripheral blood mononuclear cells (PBMCs) were treated with vital or inactivated bacteria (killed by UV-light radiation for 3 hours) from the probiotic strain *Enterococcus faecium* NCIMB 10415 (Cylactin, Cerbios) in ratios of 1:2, 2:1, 5:1, or 10:1 (PBMCs : *E. faecium*) for 1, 1.5, or 3 hours in RPMI 1640 Medium (Sigma) with l-glutamine, sodium bicarbonate, and phenol red. In addition, B-cells were isolated from 1 x 10^7 PBMCs by Magnetic Activated Cell Sorting (MACS) and immediately treated with *E. faecium* in the same ratio and under the same conditions as described above. Relative cell counts were measured by flow cytometry. Gene expression of B-cell specific genes immunoglobulin kappa constant (IGKC), immunoglobulin lambda light C region (IGLC), and cluster of differentiation 40 and 2 (CD40 and CD2) was obtained by qPCR. Statistical analysis was performed based on a Wilcoxon-Man-Whitney test.

Results: The expression of IGKC, IGLC, and CD2 in sorted B-cells isolated from blood PBMCs tended to be lower after treatment with vital bacteria for 1.5 hours compared to untreated controls. This is consistent with previous *In vivo* findings, were *E. faecium* fed pigs showed lower expression of B-cell relevant genes in ileal lymph nodes (1) indicating a more inhibitory effect of vital *E. faecium* on B-cells. In order to identify mechanisms of immune cell activation mediated by a direct mutual contact of PBMCs and *E. faecium* and to evaluate whether the bacterial surface or secreted factors are involved, experiments with vital and UV-inactivated bacteria were performed. There were higher relative cell counts of $CD21^+$ (35,2 %) and $CD79^+$ (11,1 %) B-cells after incubation with UV-inactivated *E. faecium* bacteria compared to the untreated control ($CD21^+$: 25,4 %; $CD79^+$: 8,5 %) ($p<0.05$), while there was no effect with vital bacteria for both markers. These findings suggest a lower B-cell activation and proliferation while treatment with vital *E. faecium* bacteria, which is in line with lower gene expression of B-cell specific genes and lower B-cell count after treatment. In contrast, inactivated bacteria may have an effect on B-cell activation and proliferation.

Conclusions: Hence, we propose a specific immunomodulatory effect of UV-inactivated *E. faecium* trough a specific cell surface component, which presumably effects immune response towards an enhanced B-cell answer. While this study is still ongoing, it already could provide evidence for a direct immunomodulatory effect of *Enterococcus faecium* NCIMB 10415 on B-cells, *In vitro*.

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81.

Supplementation of chestnut extract reduces severity of post-weaning diarrhoea in artificially infected piglets

Supplementierung von Kastanienextrakt reduziert den Schweregrad von Absetzdurchfällen bei künstlich infizierten Ferkeln

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Post-weaning diarrhoea (PWD) in piglets is one of the most widespread diseases in pork production (1). At weaning, digestive tract physiology is rapidly modified and piglets are more susceptible to gastrointestinal pathogens, such as enterotoxigenic *E. coli* (ETEC) F4 responsible for diarrhoea. The increased occurrence of antimicrobial resistances strongly incites the development of alternative solutions. Among the solutions, nutritional approaches could represent a good alternative. Bioactive compounds such as hydrolysable tannins are known to have antimicrobial properties (2). The present experiment aimed to study whether a standard diet (**SD**) supplemented with 2% chestnut extract (**CE**; Silvafeed Nutri P/ENC for Swine, Silvateam, Italy) containing hydrolysable tannins in piglets infected or not with ETEC F4 could impact the development of diarrhoea and digestion of piglets artificially infected with ETEC F4.

Methods: At 23 ± 3 d of age, 48 piglets susceptible to ETEC F4 were weaned and allocated in a 2 x 2 factorial design balanced for weaning body weight and litter. Piglets were housed in groups of 3 per pen. From the day of weaning, piglets had *ad libitum* access to water, electrolytes and either SD or CE diet. Diets were formulated to contain 18.5% crude protein and 14 MJ/kg DE, according to the Swiss feed recommendations for pigs. In each of the 2 groups, 4 d after weaning, 12 piglets were orally infected (**INF**) with 5 ml of ETEC (F4ac, LT+, STb+) suspension at 108 CFU/ml, whereas the remaining 12 received 5 ml PBS (**NINF**). A 4-level scale (1= dry to 4=watery diarrhoea) was used to monitor daily faecal score. The ETEC shedding and faecal dry matter were monitored by collecting swab and rectal samples. ETEC shedding was assessed by microbial culture on an Eosin-Methylene Blue agar medium supplemented with 50 µg/ml rifampicin. Four piglets per group were sacrificed 3, 6 and 7 d post-infection. Colon contents were collected to determine organic matter digestibility. Statistical evaluation was performed with SAS Software using the MIXED procedure for data of feed intake, average daily gain, faecal dry matter, ETEC shedding and organic matter digestibility and the GLIMMIX procedure for data of faecal score and percentage of piglets with diarrhoea.

Results: Infection had no effect ($P>0.10$) on average daily weight gain (127 vs 114 g/d for INF and NINF, respectively) nor on feed intake per pen (488 vs 449 g/d for INF and NINF, respectively). However, INF piglets had ($P< 0.05$) higher faecal score, lower faecal dry matter and tended ($P=0.06$) to have more diarrhoea than NINF piglets. ETEC shedding was greater ($P< 0.001$) in INF than NINF piglets. Organic matter digestibility was not affected ($P>0.10$) by infection. Feed intake per pen were similar ($P= 0.84$) between the SD and CE groups (475 vs 461 g/d for CE and SD respectively). However, piglets who received CE tended ($P=0.08$) to grow faster than piglets from the SD group (142 vs 99 g/d for CE and SD, respectively). In addition, compared with the SD diet, supplementation with CE reduced ($P< 0.05$) faecal score and percentage of piglets with diarrhoea and increased faecal dry matter. Surprisingly, diet had no effect ($P= 0.63$) on ETEC shedding. Organic matter digestibility was similar ($P> 0.10$) between the two dietary treatments.

Conclusion: Infection was able to induce diarrhoea, as depicted by the greater ETEC shedding, but had no effect on growth, feed intake or digestibility. Compared to treatment SD, supplementation with CE seemed to improve growth without affecting feed intake. This suggests that CE improved feed efficiency. As previously observed, CE diet decreased the severity of diarrhoea, which leads to a greater faecal dry matter but in the present study, ETEC shedding was not lowered. Further investigations are still needed to better understand how CE is acting in the gut.

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82.

Effect of one-time postnatal administration of immunoglobulin and trace mineral providing feed supplements on corresponding serum levels in Holstein calves during the first 14 days *post natum*

Einfluss einer einmaligen postnatalen Gabe von Immunglobulin und Spurenelement liefernden Ergänzungsfuttermitteln auf die entsprechenden Serumkonzentrationen bei Holstein Kälbern während der ersten 14 Tage post natum

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During early postnatal phase calves are highly sensitive to dietary limitations affecting their short-term growth performance and health as well as their long-term performance as mature cattle. Following an adequate supply of immunoglobulins (Ig) through colostrum of good quality for passive immunization within first hours *post natum* (*p. n.*) calves require a sufficient supply of energy and trace minerals (TM) for proper immune function and disease prevention further. However, milk-derived feedstuffs show naturally low dosages of immunologically relevant TM such as iron (Fe) and selenium (Se) fulfilling calves requirements only marginally. In this context, the present study examined additive effects of one-time administration (day 1) of Ig as well as Fe and Se providing feed supplements on corresponding serum levels of Holstein calves during the first 14 days *p. n.*.

Methods: Thirty-two female and twenty-eight male one-day-old Holstein calves were equally allocated to four experimental groups (n = 8 female and 7 male calves per each group) receiving four different dietary treatments (T1 to T4) on day 1 *p. n.*. Calves of group T1 were only supplied with colostrum (control group), whereas calves of the other groups were additionally supplied with one bag of a commercial Ig provider (provider of IgY, IgG, IgM and IgA) solved in 1 litre colostrum (group T2), one commercial TM bolus (provider of Fe, Se and vitamin E) administrated orally (group T3) and both supplements in combination (group T4), respectively. All calves were individually kept in straw-bedded calf hutches, received first colostrum including the respective treatment within the first 4 hours *p. n.* and were fed with farm-own mixed colostrum until day 3 *p. n.*. Afterwards 8 litres of milk replacer (200 g/l; 22.5% CP, 16.5 MJ ME/kg) were offered to calves via teat buckets twice a day until the end of the trial. Individual feed intake was recorded daily. At day 1 (before treatment), 7 and 14 *p. n.* calves were weighed and serum samples were taken from the jugular vein. Latter ones were analysed for glucose, Fe and Se levels via spectrophotometry (ABX Penta C400, Horiba Medical, USA) as well as for immunoglobulin IgY, IgG and IgA via ELISA kits. For statistical evaluation of data a three-factorial ANOVA with sex (female, male), treatment (T1 to T4) and age (day 1, 7 and 14 *p. n.*) as fixed effects as well as their two-factorial and three-factorial interactions was performed using SAS 9.4 procedure MIXED (2012) with repeated measures and Tukey-Kramer test as correction method. Differences were considered as significant for $p \leq 0.05$.

Results: During the entire trial calves daily colostrum and milk replacer intake as well as their daily weight gain did not differ between experimental groups. Whereas serum levels of examined TM and Ig showed no differences between groups at day 1 *p. n.* and between female and male calves throughout the entire study, these parameters responded in different ways to the dietary treatments provided. In comparison with control calves (T1), calves of groups T3 and T4 showed higher mean serum levels of Fe and Se ($p<0.001$; Table showing mean serum levels of day 1, 7 and 14 *p. n.*), whereas calves of groups T2 and T4 had higher mean serum levels of glucose and IgY. In addition, T2 calves showed higher mean serum levels of IgG and IgA than control ones ($p<0.05$).

	Glucose	Iron	Selenium	IgG	IgA
Treatment (T)	**mg/dl**	**µmol/l**	**µg/l**	**µg/ml**	**mg/ml**
T1: Control	108 ^{c}	22.2 ^{b}	52.5 ^{b}	13.4 ^{b}	0.28 ^{b}
T2: Ig	116 ^{b}	26.7 ^{ab}	56.2 ^{b}	17.7 ^{a}	0.34 ^{a}
T3: TM	112 ^{bc}	30.9 ^{a}	62.5 ^{a}	14.1 ^{b}	0.28 ^{b}
T4: Ig + TM	124 ^{a}	30.4 ^{a}	61.1 ^{a}	15.4 ^{ab}	0.31 ^{ab}
PSEM	2.20	1.53	1.15	1.08	0.01
p value: treatment	<0.001	<0.001	<0.001	<0.05	<0.001

a-c: Values with different superscripts differ significantly within the same column ($p<0.05$).

Conclusions: The present study indicated that one-time postnatal administration of Ig providing feed supplements might be a suitable approach to improve the systemic immune status of neonatal calves by elevating serum Ig levels. Because Fe and Se play a pivotal role in the immune function of calves, an additional supply of Fe and Se provided by a bolus increased the corresponding serum levels which may improve the systemic immune status of calves during early postnatal phase further.

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83.

Ex vivo-studies on survival of *Salmonella* Derby in gastric contents of pigs fed a diet without/with Benzoic acid

Ex vivo-Untersuchungen zur Überlebensrate von Salmonella Derby im Mageninhalt von Schweinen, nach Einsatz eines Mischfutter ohne/mit Benzoesäurezusatz

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According to data from the QS Salmonella Monitoring the number of pig farms has experienced a shift to category II and III over the last few years [1]. Organic acids are commonly and successfully used in compound feeds to reduce *Salmonella* in pigs [2]. To expand the repertoire of applied organic acids in pig feed this study was focused on Benzoic acid (BA), a licensed zootechnical feed additive. Stomach contents containing BA might favour the barrier function of the stomach, i. e. reducing growth of *Salmonella* Derby (*S.* Derby) as shown in a previous study [3]. Therefore, the impact of BA in porcine stomach contents on the survival rate of *S.* Derby was investigated.

Material and Methods: Conventional wheat based compound feed was offered to 22 pigs for five weeks (DM: 904 g/kg, 15.2 MJ ME/kg DM, CF: 44.1 g/kg DM, CP: 205 g/kg DM). The experimental group (+ BA, n = 11) received a conventional compound feed containing 1 % of BA (upper allowed level) whereas the control group (- BA, n = 11) received the same diet without the BA supplementation. All animals (age: 63 ± 1 days old) were euthanized, gastric contents were collected and frozen (-20°C) until the day of examination. Each gastric content was thawed, homogenized and divided into five plastic bags with each 12g (- BA1-5 and + BA1-5). All five aliquots of -/+ BA were simultaneously inoculated with *S.* Derby (Ø 8.16 ± 0.282 log10 cfu/g) and placed in a water bath for incubation at 37 °C. After 0, 60, 120, 240 and 360 minutes of storage 2g of each aliquot -/+ BA were removed for measuring the pH-value. The remaining 10g were analysed for log10 cfu *S.* Derby/g gastric content by MPN-technique. Statistical analyses were performed in SAS® software (Cary, NC, USA) by using Wilcoxon and ANOVA.

Results: The counts of *S.* Derby per g gastric content did not differ significantly during 360 min of incubation between the groups. The pH-value of gastric content was not influenced by BA supplementation (Tab. 1). In both groups at lower pH lower cfu *S.* Derby/g gastric content were found, but Ba supplementation did not affect the counts of *S.* Derby.

Table 1 Relationship between gastric-pH [x] and counts of *S.* Derby (log_{10} cfu/g gastric content) [y], $p < 0,05$

Incubation	min	0	60	120	240	360
Compoundfeed[- BA]	$\bar{x}$	4.06± 1.48	4.10± 1.49	4.08± 1.46	4.02± 1.36	3.93± 1.30
	$\bar{y}$	6.87± 1.14	5.39± 3.17	5.51± 3.08	5.47± 3.86	6.18± 3.23
Compoundfeed[+ BA]	$\bar{x}$	4.39± 0.926	4.41± 0.917	4.51± 0.982	4.40± 0.922	4.39± 0.905
	$\bar{y}$	7.23± 0.764	6.11± 1.99	5.95± 2.15	5.88± 2.03	5.76± 2.38

Conclusion: The effects in earlier *ex vivo* studies [3] of lowering the gastric-pH and reducing *S.* Derby in gastric content by adding BA to the gastric content in an incubation container could not be confirmed by this trial. *Salmonella* were exposed only to the residual amounts of BA that were still present in the stomach. At three hours post prandial only 15.5 to 44.6 % of BA of the initial dose were still present in gastric contents (unpublished data). The counts of *S.* Derby in gastric content were predominately reduced by gastric-pH rather by the concentration of BA which was investigated in this trial (initially 1 % in compound feed). The ex vivo exposure of GIT contents to feed additives seems to be a suitable model to test distinct conditions in the digesta on diverse parameters of interest.

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84.

Methylmalonic acid concentrations in serum as an indicator of the vitamin B12 status in pigs

Die Methylmalonsäure-Konzentration im Serum als Indikator für den Vitamin B12-Status beim Schwein

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Vitamin B12 (Vit. B12) plays an important role in amino acid metabolism, nucleic acid synthesis and methyl-group transfer, and is therefore an essentiell component for genomic stability. Assays for the measurement of serum Vit. B12 and homocysteine (a Vit. B12-dependent metabolite) are available for pigs, however those measurements do not accurately determine the Vit. B12 status in pigs (1). Serum Vit. B12 is not a direct reflection of the intracellular Vit. B12 status and homocysteine, a product of the malfunctioning of the intracellular enzyme methionine synthase, has more than one cofactor (e.g., Vit. B6 and B9). In veterinary medicine, decreased Vit. B12 concentrations are associated with increased methylmalonic acid (MMA) concentrations in serum (2). Increased serum MMA concentrations can result from a malfunction of methylmalonic acid-CoA mutase, a Vit. B12-dependend enzyme. Changes in the serum MMA concentration reflect the intracellular availability of Vit. B12. Thus, measurement of MMA may help to prevent deficiencies and associated diseases and may be a valuable tool to evaluate the dietary requirements for Vit. B12 in pigs. Currently, no large data sets are available that compare serum Vit. B12 concentrations with serum homocysteine and MMA concentrations in pigs. This study aimed to evaluate serum MMA concentrations in several groups of pigs, with the hypothesis that MMA concentrations show a stronger association with Vit. B12 than homocystein concentrations.

Methods: Data from three unpublished studies were used. Serum samples from A) 12 pigs were collected at week 6, 7, 8, 9, 10, 14, 18, 22, and 26 of life. B) 32 pigs from a selected farm with a history of confirmed *L. intracellularis*-infections. Serum samples were collected on day 0, 7, 14 and 21 of 7-weeks old pigs. C) 14 age-matched pigs that were randomly allocated to 3 groups (controls [n=4], experimentally-induced exocrine pancreatic insufficiency (EPI) in 7-weeks old pigs [n=5], and experimentally-induced EPI in 16-weeks old pigs [n=5]). Serum samples were obtained from all pigs at 9, 15, 21, and 26 weeks of age. In all studies, the pigs received a diet according to the national dietary recommendation (NRC 1998, UFA 2014 and GfE 2008, respectively), where the study was conducted. Serum Vit. B12, homocysteine, and MMA concentrations were measured in all samples using a electro-chemiluminescence immunoassay, homocysteine enzyme-cycling assay, and high-performance-liquid-chromatography, respectively. Depending on the distribution of the data in each study, a Pearson or Spearman rank sum correlation coefficient (ρ) was used to test for a correlation between serum Vit. B12, homocysteine, and MMA concentrations.

Results: For samples from pigs from study A), a moderate negative correlation was observed for serum Vit. B12 and homocysteine (ρ: -0.31 [95%CI: -0.47 to -0.12]; p=0.0013) as well as serum Vit. B12 and MMA concentrations (ρ: -0.21 [95%CI: -0.39 to -0.01]; p=0.0290). For samples from pigs from study B) and C), a moderate to strong negative correlation was present between serum Vit. B12 and MMA concentrations (ρ: -0.40 [95%CI: -0.54 to -0.23]; p<0.0001 and ρ: -0.85 [95%CI: -0.91 to -0.76]; p<0.0001, respectively) but not for serum Vit. B12 and homocysteine (ρ: -0.09 [95%CI: -0.27 to 0.08]; p=0.2950 and ρ: -0.09 [95%CI: -0.35 to 0.19]; p=0.5131, respectively). An association between both Vit.B12-depentent metabolites (homocysteine and MMA) was observed in study A) ρ: 0.49 [95%CI: 0.33 to 0.63]; p<0.0001 and study B) ρ: 0.24 [95%CI: 0.06 to 0.40]; p=0.0068, but not study C) ρ: 0.07 [95%CI: 0.20 to 0.33]; p=0.6064.

Conclusion: As hypothesized these studies suggest that the measurement of MMA, a Vit. B12-dependent metabolite, in serum holds promise to improve the determination of the Vit. B12 status in both, healthy and diseased pigs. Further investigations are warranted to evaluate the dietary requirements of Vit. B12 in pigs based on serum MMA concentrations.

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85.

Effects of lactic acid treated by-products in a diet supplemented or not with inorganic P on ruminal fermentation and nutrient degradation *In vitro*

Effekte einer milchsäurebehandelten Nebenproduktmischung mit oder ohne anorganischem P Zusatz auf die ruminale Fermentation und Abbaubarkeit der Nährstoffe In vitro

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Previously the treatment of barley with lactic acid (LA) increased mineral solubility and enhanced ruminal fibre degradation (1,2). An increased availability of Phosphorous (P) might result in improved ruminal fermentation and reduction of inorganic P supplementation. Since the global supply of industrial food by-products as feedstuffs is tremendously increasing, the aim of this study was to evaluate the effects of LA treatment of a by-product mixture with or without supplementation of inorganic P on ruminal fermentation and nutrient degradation In vitro using the rumen simulation technique (RUSITEC).

Methods: The by-product mixture consisting of wheat bran, corn gluten feed, sugar beet pulp and sunflower cake was soaked either in distilled water or in 5% (vol/vol) LA solution both in a ratio of 1:5 (wt/vol) for 24h and dried at 40 °C for 96 h. A total of 4.8 g by-product mixture was incubated in RUSITEC in nylon bags (150 µm mesh size) together with 5 g (DM) forages (silage and ventilation dried hay both from first cut in equal amounts (on DM basis), and 0.2 g minerals (free from inorganic P (P-) or with inorganic P (P+) from Monocalciumphosphate) in two experimental runs each lasting 10 days. Forages were previously air dried at room temperature and milled passing a 3.14 mm sieve. The adaptation period lasted from day 1-5 and sampling period started on day 6. Inorganic P supplementation accounted for 16 mg P. In addition, buffer for the RUSITEC system provided 284 mg inorganic P (Na_2HPO_4*$2H_2O$) per fermenter and day for P+ diets. P- diets were infused with a P free buffer. Samples were collected for short chain fatty acids (SCFA), ammonia and nutrient degradation analyses. Statistical analysis was done with procedure mixed (SAS 9.4) considering fixed effects of LA, inorganic P supplementation and their two-way interaction, whereas fermenter and experimental run were considered as random effects.

Results: Lactic acid increased ($P < 0.001$) total SCFA concentration and shifted SCFA proportions ($P < 0.001$) towards propionate. This treatment also lowered CP degradation ($P = 0.001$) and ammonia concentration ($P < 0.001$). Fiber degradation remained unaffected by LA. Inorganic P supplementation reduced ($P < 0.001$) total SCFA concentration and proportion of acetate but not propionate and butyrate. Furthermore, P supplementation decreased CP degradation ($P = 0.002$) and ammonia concentration ($P = 0.029$), as well as ($P = 0.043$) NDF degradation and gas production. However, P supplementation increased ($P = 0.047$) degradation of non-fiber carbohydrates (NFC). Since (1) detected only negligible amounts of lactate in fermenter fluid when treating barley with 5% lactic acid solution which gave rise that lactate is easily metabolizable for microbiota we expected similar findings for the study in question. Throughout the trial pH and redox potential were maintained constant.

Conclusions: Lactic acid treatment of by-products enhanced propionate formation and may increase the net duodenal supply of protein for the host. Under conditions of this study (diets rich in total P and fiber), inorganic P supplementation hampered fibrolytic ruminal activity but enhanced degradation of NFC.

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This study was funded by BMLFUW, Land Salzburg, Raiffeisen Salzburg and Werner Lampert Beratungsges. m. b. H.

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86.

Increasing concentrations of a grape extract in broiler diets: Impact on histology and antioxidative status in the ileum

Ansteigende Traubenextraktkonzentrationen im Broilerfutter: Einfluss auf Histologie und antioxidativen Status im Ileum

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Inclusion of by-products of winery in animal nutrition is interesting due to high polyphenol (PP) content. The polymeric PP predominantly have pre-absorptive properties, interacting with proteins and morphology, reducing the absorptive capacity and performance in high concentrations. Post-absorptive properties of mono- and dimeric PP are the enhancement of antioxidative properties in epithelial and peripheral tissues (1). Due to low absorption rate, PP may concentrate in digesta of distal small intestine, therefore intensifying potential effects. As part of a large project, the hypothesis is that a certain dietary concentration of grape extract (GEx) would improve the ileal antioxidative properties, without impairing performance. whereas high contents have an inhibitory effect on the villi growth, reducing nutrient absorption.

Methods: 432 one day old broiler chicks (Ross 308) were equally distributed to 6 feeding groups (6 replicates) fed increasing concentration of a dry GEx (0; 0.005; 0.01; 0.1; 1; 2 g/kg; Nor-Grape 80, Nor-Feed, France). A starter (d1-d14; 12.4 MJ AME_N/kg, 220 g CP/kg), grower (d15-d28; 12.7 MJ AME_N/kg, 210 g CP/kg) and a finisher diet (d29-d38; 12.8 MJ AME_N/kg, 189 g CP/kg) were provided *ad libitum*. Two representative broilers per pen were slaughtered at the end of trial for distal ileal tissue sampling. Samples were embedded in paraffin and sections stained with AB-PAS for histometric measurements. Villus height to crypt depth ratio (VC ratio) was calculated. Goblet cells were counted as number of cells/200 µm of villus. Activity of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase was determined. Total antioxidative capacity (TAC), as the concentration of ABTS (2,2‘-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), as Trolox equivalents), and thiobarbituric acid reactive substances (TBARS) were analysed. Statistical analyses were performed using the procedure GLM of SAS 9.4. with the effects GEx, sex and their interactions. Polynomial contrasts (linear, quadratic and cubic) were additionally calculated.

Results: Ileal crypt depth showed a u-shaped relation with lower values in medium GEx concentrations. Likewise, number of goblet cells in villi was higher in medium GEx concentrations (quadratic contrast $p<0.05$). Furthermore, 0.005g GEx/kg tended to increase VC ratio compared to 2 g/kg. Activity of antioxidative enzymes was not affected by dietary treatment. Both TAC and lipid peroxidation tended to linearly increase with increasing dietary GEx concentration.

g GEx/ kg as fed	0	0.005	0.01	0.1	1	2	SEM	L	Q	C
Villus height, µm	491	570	505	535	490	517	11	ns	ns	ns
Crypt depth, µm	109	104	112	104	101	112	2	ns	*	ns
VC ratio	$4.77^{(ab)}$	$5.78^{(a)}$	$4.93^{(ab)}$	$5.41^{(ab)}$	$5.29^{(ab)}$	$4.58^{(b)}$	0.13	ns	ns	ns
Goblet cells, n/200µm	26.1	26.4	25.5	27.1	32.7	26.6	0.7	ns	*	ns
GPx, U/g protein	29.4	25.3	27.0	26.0	26.1	27.5	0.7	ns	ns	ns
Catalase, U/mg protein	20.7	21.3	23.1	20.9	21.6	23.2	0.7	ns	ns	ns
SOD, U/mg protein	5.72	5.94	6.67	6.28	6.76	5.71	0.19	ns	ns	ns
ABTS, µmol Trolox eq/g FM	83.7	82.6	82.3	81.2	84.6	86.2	0.8	(*)	ns	ns
TBARS, nmol/g FM	221	230	240	217	247	270	9	(*)	ns	ns

L=linear; Q=quadratic; C=cubic; *=p<0.05; (*)=p<0.10; ns=p>0.10; values differing in superscripts: p<0.10

Conclusions: Even though we observed reduced performance when feeding high GEx concentrations, as shown by Schabelreiter et al. (2), there was only a trend of affected villus-crypt architecture in the ileum, indicating reduced absorptive capacity. The concomitant increase in TAC and TBARS points to a prooxidative action of high GEx concentrations in the ileum. No dietary concentration of GEx can be defined, improving oxidative stability without affecting performance.

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87.

Effects of lignocellulose supplementation on performance, intestinal inflammation and the gut microbiome in broilers

Effekte der Supplementierung mit Lignocellulose auf Leistung, Entzündungsgeschehen und Mikrobiom im Darm von Broilern

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Broilers have a certain requirement for fibre in their diet (1). Although indigestible for endogenous animal enzymes, dietary insoluble, but fermentable fibres can help to ensure the functioning of the digestive tract due to physical effects, are potential substrates for microbial fermentation in the hindgut (1), and may have anti-inflammatory effects in the intestine. Therefore, we investigated the effects of a partly fermentable insoluble lignocellulose in comparison to a diet supplemented with a non-fermentable lignocellulose and a non-supplemented control diet.

Methods: 96 day-old male Cobb500 broiler chickens with 41.6±3.2 g body weight (mean ± SD) were allotted to 12 cages with each 8 chicks, and grouped into 3 experimental groups. The broilers were phase-fed three maize-wheat-soybean meal-based basal diets during days 1-10 (6.7% CF), 11-21 (5.2% CF), and 22-35 (5.0% CF) (control group), or these diets supplemented with 0.8% of a standard lignocellulose (FibreCell, Agromed Austria GmbH, Kremsmünster, Austria) (77.3% CF, 90.5% NDF, 77.7% ADF) (group LCS) or 0.8% of a eubiotic lignocellulose product (OptiCell, Agromed Austria) (77.4% CF, 89.9% NDF, 77.4% ADF) with similar chemical composition, but higher susceptibility to microbial fermentation (group LCF). Body and carcass weights and feed consumption were recorded after 35 days. Samples of jejunum, colon, and cecum mucosa were collected and stored at -80°C until quantification of transcript levels of the pro-inflammatory genes interleukin 1b (IL1b), IL2, IL6, IL8, IL17, lipopolysaccharide induced TNF factor (LITAF), and vascular cell adhesion molecule 1 (VCAM1) by RT-PCR (2). Cecum digesta was stored at -80°C until analysis of short chain fatty acids (SCFA) (2) and the composition of the bacterial population by 16S rRNA Gene sequencing (3). One way ANOVA was performed with treatment as fixed factor.

Results: Feed intake (3459±244, 3386±149, and 3452±117 g (as is) in control, LCS and LCF groups; P=0.82), body weight gains (2390±163, 2329±112, and 2448±70g; P=0.56), and the feed:gain ratio (1.42±0.05, 1.42±0.05, and 1.36±0.05; P=0.21) were similar among groups. The dressing percentage was higher in broilers fed LCF-supplemented diets (72.1±1.2%) compared to the control group (70.4±1.9%; P=0.028), group LCS being intermediate (71.4±1.5%). Supplementation of LCF, but not of LCS, decreased relative transcript levels of IL1b (P=0.018), IL17 (P=0.050), IL2 (P=0.054) and IL8 (P=0.093) compared to the control group in the jejunum mucosa, whereas those of IL6, LITAF and VCAM were similar among groups. In the cecum and colon mucosa, cytokine expression was similar among groups. In the cecum digesta, total SCFA concentrations did not differ between groups (P=0.46). Molar proportions of acetic acid were higher in group LCF (78.6±1.8%) compared to the control (74.3±3.4%) and LCS (75.8±2.4%) group (P=0.001), and those of butyric acid were lower in group LCF (15.0±2.4%) compared to control (19.1±3.7%) and LCS (18.4±2.7%) group (P=0.003). Concomitantly, the cecal bacterial population differed among groups. Supplementing diets with LCF was associated with a lower abundance of Firmicutes compared to the control group (96.2 vs. 97.6% of total sequences; P=0.08), and within, of Ruminococcaceae (15.8 vs. 18.3%; P=0.028) and Lactobacillaceae (2.88 vs. 5.80%; P=0.046). In contrast, in group LCF compared to the control group, higher abundance of Peptostreptococcaceae (10.34 vs. 4.74%; P=0.001), Clostridiacae (0.21 vs. 0.01%; P=0.004), Erysipelotrichaceae (4.56 vs. 2.46%; P=0.035), and Proteobacteria (3.50 vs. 2.03%; P=0.09) was found. However, LCS supplementation did not affect the bacterial population compared to the control group.

Conclusion: The dietary supplementation of the partly fermentable lignocellulose product OptiCell, but not of FibreCell, exerted anti-inflammatory effects in the small intestine and modulated the bacterial population and SCFA generation in the cecum. These changes may have contributed to the increased dressing percentage in LCF-supplemented broilers at slaughter.

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88.

Effects of lignocellulose product containing lignans on performance and gut morphology in broilers

Einfluss eines lignanhaltigen Lignocelluloseproduktes auf die Leistung und die Darmmorphologie beim Broiler

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Introduction: Lignans are a class of polyphenols which is widely distributed throughout the plant kingdom. They are present in high amounts in bark of special trees used in Traditional Chinese Medicine and have been reported to show anti-inflammatory, anti-microbial and antioxidant activity (1), offering potential for use as an alternative for antibiotic growth promotors (AGPs). Aim of the trial was to test a lignocellulose product containing lignan-rich bark (dry, powdered premix based on lignocellulose from lignan-rich bark; agromed® ROI; agromed Austria GmbH, Kremsmünster), compared to a commercial feed AGP under trial conditions in Brazil.

Methods: 416 one day old male broilers (Cobb 500) were randomly allocated to 4 treatments with 8 repetitions of 13 birds each. Treatments were NC – negative control (standard broiler diet based on corn, soybean meal); PC – positive control (NC + 16.5 mg/kg Virginiamycin); NC+LP (NC + lignocellulose product); PC+LP (PC + lignocellulose product). LP (lignocellulose product; premix based on lignocellulose from lignan-rich bark; agromed® ROI; agromed Austria GmbH, Kremsmünster, Austria) was supplemented at a dosage of 400 mg/kg feed. Broilers were fed mashed starter (day 1-21), grower (day 22-33) and finisher (day 34-42) diets according to Brazilian standards (2). Feed intake, weight gain, and feed to gain ratio were recorded at day 7, 21, 35 and 42. At 21 days of age, one bird per replicate was sacrificed to collect intestinal mucosa samples for determination of villi length. At 42 days, two birds per replicate were removed to evaluate the carcass characteristics. Carcass yield (relative to live weight of poultry), and yield of breast (boned and without skin) and legs (chicken thigh and chicken drumstick) were evaluated in relation to the weight of the eviscerated carcass, without feet and without head. The data were analysed by the SAS statistical program using the Tukey Test.

Results: LP supplementation significantly ($p<0.005$) improved weight gain, feed conversion, carcass and breast percentage and villi height(table 1) compared to both control groups. Addition of Virginiamycin (PC) resulted in reduced feed conversion, breast yield and improved carcass proportion compared to the negative control.

Table 1: Performance data, carcass characteristics and villi length of experimental groups

	NC	**PC**	**NC + LP**	**PC + LP**
Weight gain d 1-42, g	2,584^{c}	2,585^{c}	3,090^{a}	3,034^{b}
Feed to gain ratio d 1-42, g:g	1.71^{d}	1.68^{c}	1.46^{a}	1.52^{b}
Carcass, %	67.8^{d}	68.6^{c}	68.9^{b}	69.6^{a}
Breast, %	31.7^{c}	31.1^{d}	33.5^{a}	32.5^{b}
Villus height, µm	747^{c}	838^{bc}	963^{a}	925^{ab}

a, b, c, d values with different superscripts within a row differ significantly ($p<0.005$)

Conclusion: The results of this study indicate that supplementation with LP supports the performance of broilers. The addition of LP resulted in higher villi height when compared to the NC and PC treatments. The improvement for this characteristic is in agreement with the performance data. The lignan-containing product offers a potential as an alternative to AGPs, taking into account that the performance data in the LP-groups was improved either compared to the NC as well as to the PC. However, the mode of action of LP has to be clarified in further studies.

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89.

Modulation of the plasma metabolomic profile in cows challenged with concentrate-rich diets supplemented with phytogenic compounds or autolyzed yeast

Modulation des Plasma Metaboloms von Milchkühen durch kraftfutterreiche Fütterung und Zusatz phytogener Komponenten oder autolysierter Hefe

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The feeding of high-grain diets increases the risk of subacute ruminal acidosis (SARA) and might induce perturbations of the systemic metabolism. Compared with single metabolite techniques, metabolomic technologies enable the detection of multiple classes of metabolites that reflect changes of key metabolic pathways. This can advance our understanding of the mechanisms behind disorders, such as SARA. Recently phytogenic compounds (PHY) and autolyzed yeast (AY) showed potential to counteract negative consequences of high concentrate feeding, by increasing the reticular pH and modulating the fermentation profile and microbial community structure (1,2). The aim of this study was to use a targeted ESI-LC-MS/MS-based metabolomics approach to describe changes in the metabolome of cows experiencing SARA, whereby a special focus was put on possible modulatory effects of the addition of feed additives. We hypothesized that feed additives might reduce negative effects of high-grain feeding by counteracting SARA-induced perturbations in the systemic metabolism.

Methods: Eight ruminally-cannulated non-lactating Holstein cows were arranged according to an incomplete 4 x 3 Latin square design with 4 runs and 3 treatment groups. The treatments consisted of a concentrate-mix containing PHY (Digestarom® Dairy, BIOMIN Holding GmbH), AY (Levabon® Rumen E, BIOMIN Holding GmbH) or no additive (CON). PHY consisted of a mixture of herbs, spices, essential oils and nonvolatile extracts. During each run cows were switched from a pure forage diet (FD) to an intermittent concentrate challenge with a forage:concentrate ratio of 35:65 for one (SARA1) or two weeks (SARA2). In between cows received a pure forage diet for one week (Recovery). Reticular pH was measured continuously using wireless sensors (eCow, Dekon, UK). During FD and SARA1 blood samples were collected. A targeted ESI-LC-MS/MS-based metabolomics approach was carried out on the plasma samples. Statistical analysis was conducted using SAS (PROC MIXED), with the feeding phase and treatment groups, as well as their interactions as fixed effects, while run and cow nested within the square were considered as random effects.

Results: The first concentrate-challenge (SARA1) caused a strong decrease in reticular pH <6.0 (on average 336 min/d), differing from all other feeding phases (FD: 8 min/d, Recovery: 37 min/d, SARA2: 192 min/d; $P < 0.01$). This was accompanied by a shift in the plasma metabolome, such as a decrease in phosphatidylcholines, lysophosphatidylcholines and sphingomyelines ($P < 0.01$). Also, several amino acids and acylcarnitines were reduced in cows experiencing SARA ($P < 0.01$), whereas the effects on biogenic amines were less pronounced. Cows receiving feed additives showed lower duration of reticular pH <6.0 during SARA1 (284 min/d for PHY and 308 min/d for AY vs. 416 min/d for CON; $P \leq 0.05$). Supplementation of the diet with PHY caused an increase in several amino acids, phosphatidylcholines, and acylcarnitines compared to CON ($P < 0.05$). For AY a decrease in sarcosine, alpha-aminoadipic acid, and glutamine compared to the CON group was noticed, whereas tryptophan, isovalerylcarnitine and hydroxytetradecenoylcarnitine were increased ($P < 0.10$).

Conclusions: Data suggest that a concentrate-induced SARA was associated with strong effects on the plasma metabolome. The supplementation of PHY and AY influenced the metabolome during high concentrate feeding. Thus, feed additives may reduce negative effects of high-concentrate feeding by counteracting SARA-induced perturbations in the systemic metabolism.

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This study was funded by the Austrian Research Promotion Agency (FFG) under the scope of the COMET program.

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90.

Effects of Masson Pine pollen (*Pinus massoniana*) on cytokine gene expression in HD11 chicken macrophages *In vitro*

Zum Einfluss von Pollen der Masson Pinie (Pinus massoniana) auf die Expression von Zytokingenen in HD11 Hühner-Makrophagen In vitro

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Masson Pine pollen (*Pinus massoniana*) (MP) had positive effects on gut health of rats and pigs (1-3). However, the mode-of-action by which MP interact with the gastrointestinal system is absolutely unclear. To further highlight potential effects on the gastrointestinal immune system, this study was focussed on the effects of MP on cytokine gene expression in chicken macrophages *In vitro*.

Methods: For the *In vitro* studies, 12 MP samples from different production sites and harvest years as well as a certified Escherichia coli lipopolysaccharide standard material (LPS) (E. coli O111:B4, Invivogen) were used. HD11 chicken macrophages (4) were cultivated in a RPMI media containing 1% Glutamax, 8% fetal calf serum, 2% chicken serum and 1% penicillin+streptomycin (Thermo Scientific). Successfully cultivated cells were incubated for 6h either with a MP sample (incubation of 1 mg/mL media overnight; filtration of media prior to usage for cell cultures) or LPS. Furthermore, non-stimulated cells were used as a negative control (NC). All *In vitro* experiments were carried out in triplicate. After 6h, cells were harvested and total RNA was extracted, treated with DNase and 400 ng of each sample were used for cDNA synthesis. Data was subject of two-way ANOVA (treatment, replicate). Data analysis and presentation occurred as Log10(2-ΔΔCt).

Results: The genes coding for interleukin (IL) 8, 1ß, 6 and 12ß were upregulated by ~2, ~1.7, ~2.3 and ~1.3 log-orders of magnitude, respectively, in MP and LPS treated cells, in each case relative to NC ($P < 0.0001$) (Table).

Table Relative Interleukin (IL) 8, 1β, 6 and 12β gene expression (Log10(2-ΔΔCt)) in HD11 macrophages

	MP	LPS	NC	SEM	PTreatment
IL8	2.13[a]	2.01[a]	0.00[b]	0.14	<0.0001
IL1β	1.69[a]	1.65[a]	0.00[b]	0.11	<0.0001
IL6	2.39[a]	2.26[a]	0.00[b]	0.17	<0.0001
IL12β	1.31[a]	1.27[a]	0.00[b]	0.17	<0.0001

Notes: SEM, standard error of means, means not sharing a common superscript differ at $P \leq 0.05$.

Conclusion: Macrophages upregulated proinflammatory cytokines in the presence of MP and LPS, respectively. This indicates that MP might be able to interact with toll-like receptors at the cell surface, which induces intracellular signaling cascades at the end of which cytokine gene expression is stimulated. This may increase these cells capacity to intercept bacterial cells at the gut mucosa and might explain positive *In vivo* effects, which have been described earlier (1-3). Indeed, follow-up investigations must provide a precise chemical characterisation of the MP matrix. Thereby, it may be possible to identify molecular motifs that may interact with cell surface receptors.

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This study has been generously supported by Yantai New Era Health Industry Co. Ltd. and WZP Gmbh.

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91.

Phytase and phosphorus effects on the microbial ecology in the ileum of broiler chickens

Einflüsse von Phytase und Phosphor auf die mikrobielle Ökologie im Ileum von Broilern

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The principal storage form of phosphorus (P) in plants, the phytate, is a complex substrate which cannot be easily assimilated by broiler chickens, diminishing the availability of nutrients in the gastrointestinal tract (GIT) [1]. Thus, it is common to supply mineral feed phosphates and phytase enzymes to meet the requirement for P. Chemical properties of the content of the GIT are modified due to the mode of action of phytase, P and Ca supplements, which must affect the microbial composition [2]. Studies had reported that disappearance of phytate is low in the crop when diets did not include phytase, while upon passage of digesta to the terminal ileum reached a disappearance near to 60% [1]. Here we aim to compare the effect of two different phytase products and a mineral P source on the microbial composition in the ileum of broilers.

Methods: Twelve experimental diets were provided to a total of 864 broilers (Ross 308) distributed in 72 pens with 12 birds each. Diets were corn-soybean-based and the treatments consisted of the basal diet (4.5 g/kg of P and 6.8 g/kg of Ca) and supplementations based on four concentrations of dicalcium phosphate (DCP) (0.6, 1.2, 1.8 and 2.4 g/kg), four concentrations of Natuphos E (125, 250, 500 and 750 FTU/kg) and three concentrations of Natuphos (250, 500 and 1000 FTU/kg). On day 21, birds were euthanized by carbon dioxide following anesthesia in gas mixture. Ileum samples were collected, pooled on pen basis (n=12/pen) and stored at -80°C. Feed consumption and weight gain were recorded. For microbiota analysis, a total of 72 samples (6 replicate pens per diet), were extracted with a commercial DNA extraction kit and then subjected to Illumina 16S rRNA gene amplicon sequencing [3]. Phylogenetic analysis was assessed using Mothur, followed by multivariate statistical analysis which includes PERMANOVA routine, based on Bray-Curtis similarity index calculated from the total microbial abundance of each operational taxonomic unit (OTU) across all the samples [3].

Results: The supplementation of both enzymes increased bird performance in comparison to the basal diet, however was the highest dose of DCP the one that resulted in the highest performance values ($p \leq 0.05$). The microbial community showed statistical differences between Natuphos and DCP diets and Natuphos and basal diet ($p = 0.04$). The OTUs responsible for the fluctuations were assigned to the genus *Lactobacillus*, which was predominantly abundant in all diets (97-87%). Between them, mainly positive correlations were found when Natuphos E was included in the diet. In terms of abundances, the highest across all treatments were registered for *Lactobacillus crispatus* with 42% for the Natuphos 250 and *L. gallinarum* with 45% for Natuphos 1000, those species were negatively correlated in both diets. *L. salivarius* and *L. crispatus* (24 and 38%, respectively), were contributing to the differences between the DCP high dose and the basal diet and Natuphos 250. Furthermore, an OTU from the genus *Streptococcus* was only found in the treatment with high supplementation of DCP (2.4 g/kg), with an average abundance of 5%. Predicted functions showed significant differences between the diets in this phytase challenge, where a group including the basal diet and Natuphos at two levels (250 and 500 FTU/kg) clustered apart from the remaining diets. Functions related to nitrogen metabolism and methane metabolism are predicted to be more prevalent in Natuphos 250 and 500 compared to the other diets.

Conclusions: Known functions of DCP and phytase supplements on growth and P utilization of broilers may partially be mediated by changes in the abundance of specific bacterial species in the digestive tract.

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92.

Effect of phytogenic compounds or autolyzed yeast on the concentration of biogenic amines in the rumen fluid of cows challenged with concentrate-rich diets

Auswirkungen phytogener Komponenten oder autolysierter Hefe auf den Gehalt an biogenen Aminen im Pansensaft von Milchkühen bei kraftfutterreicher Fütterung

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Subacute ruminal acidosis (SARA) is a critical digestive disorder of cattle. SARA may result in alterations within the ruminal microflora and accumulation of toxic substances, such as biogenic amines, with severe consequences for the host`s health (1). Recently, phytogenic compounds (PHY) and autolyzed yeast (AY) showed a potential to counteract negative consequences of high-concentrate diets, such by increasing the reticular pH and modulating the microbial community structure (2,3). The aims of this study were to investigate changes in the concentration of biogenic amines in the rumen fluid of cows experiencing SARA and to test whether it is possible to counteract their increase by the addition of feed additives.

Methods: Eight ruminally-cannulated non-lactating Holstein cows were subjected to 3 different treatments according to an incomplete 4 x 3 Latin square design with 4 runs. The treatments differed in the supplementation with feed additives. Cows of the control (CON) group received no additive, while the other two groups received either PHY (Digestarom® Dairy, BIOMIN Holding GmbH) or AY (Levabon® Rumen E, BIOMIN Holding GmbH) via concentrate. The AY (*Saccharomyces cerevisiae)* contained mannans, glucans, peptides and amino acids while PHY consisted of a mixture of herbs, spices and essential oils. To simulate intermittent dietary acidotic conditions the cows were switched from a pure forage diet (Baseline) to an intermittent SARA challenge with a forage:concentrate ratio of 35:65 (DM basis) for one (SARA1) or two weeks (SARA2). Forage (50% DM) and concentrate (90% DM) were offered separately. In between SARA1 and 2 cows received the pure forage diet for one week. During Baseline, SARA1 and SARA2 samples from the particle-associated rumen liquid were taken once a day 8 h after morning feeding and analyzed for the concentration of biogenic amines using HPLC. Statistical analysis was conducted using ANOVA, taking repeated measurements on the same cow within one run into account.

Results: SARA1 caused a stronger decrease in reticuloruminal pH compared to SARA2, which was accompanied by a more pronounced change in the concentration of biogenic amines in the rumen fluid. Overall, a 2-fold increase in the concentration of biogenic amines was found, with histamine showing the strongest increase reaching a 7.4-fold concentration in SARA 1 compared to the Baseline ($P < 0.01$). The increase in ethanolamine, pyrrolidine, cadaverine, and histamine was more pronounced in SARA 1 compared to SARA 2. Supplementation of the ration with PHY resulted in a decreased concentration of pyrrolidine (-34%), histamine (-69%) and spermidine (-35%) in SARA 1 and a lower concentration of cadaverine (-50%) in SARA 2 compared to CON ($P \leq 0.09$). For AY a decrease in ethanolamine (-21%), methylamine (-43%), histamine (-40%) and spermine (-80%) in SARA 1 compared to the CON-cows was observed ($P \leq 0.08$).

Conclusions: Data suggest that the induction of SARA caused strong effects on the concentration of biogenic amines in the rumen fluid. Furthermore, PHY and AY showed potential to counteract this increase likely due to their increasing effect on reticular pH and effects on the rumen microbiome (2,3). Thus, feed additives reduced negative effects of high-concentrate feeding by counteracting SARA-induced elevation of toxic substances, especially when a stronger decrease in ruminal pH occured.

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This study was funded by the Austrian Research Promotion Agency (FFG) under the scope of the COMET program.

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93.

Inflammasome signaling in porcine monocyte-derived dendritic cells after stimulation with probiotic *E. faecium* and enterotoxigenic *E. coli*

Induktion des Inflammasom-Signalweges in porzinen Blutmonozyten-abgeleiteten dendritischen Zellen nach Stimulation mit probiotischen E. faecium und enterotoxischen E. coli

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Dendritic cells (DC) detect luminal antigens, e.g., originating from intestinal microbiota and are essential for maintaining the immunological balance in the gut (1). Microbial molecular patterns can be detected by intracellular pattern recognition receptors, such as NLRP3 (nucleotide oligomerization domain-like receptor protein 3) inflammasomes. Inflammasomes are implicated in the pathogenesis of inflammatory intestinal diseases (2). Furthermore, evidence suggests that probiotic bacteria are able to influence the inflammasome pathway. During an enterotoxigenic *Escherichia coli* (ETEC) infection *In vitro*, *Enterococcus* (*E.*) *faecium* NCIMB 10415 strengthened the intestinal barrier and attenuated the proinflammatory response in porcine intestinal epithelial cells (3). Therefore, the aim of the study was to analyze the ability of a pathogenic ETEC strain with relevance to post-weaning diarrhea to induce inflammasome activation in porcine monocyte-derived DC (MoDC) and to investigate whether *E. faecium* modulates the inflammatory response towards the enteropathogen.

Methods: Peripheral blood mononuclear cells were purified from whole blood samples from 9 pigs by density gradient centrifugation. Monocytes were positively selected by magnetic-activated cell sorting by using CD14 MicroBeads. In a six-day protocol, MoDC were generated from CD14+ monocytes by adding recombinant porcine granulocyte-macrophage colony stimulating factor and interleukin (IL)-4.

For inflammasome activation, immature MoDC were stimulated with either a probiotic *E. faecium* strain or a pathogenic ETEC strain. In a coincubation setup, cells were preincubated with the probiotic for 2 h and subsequently challenged with ETEC. After 2 h of bacterial exposure, cells were supplemented with gentamicin-containing medium in order to avoid bacterial overgrowth. Samples were taken 6 h or 20 h after bacterial addition. At the mRNA level, the inflammasome components IL-1β, IL-18, NLRP3, and caspase-1 were studied by quantitative real-time PCR. The protein release of IL-1β was determined in cell culture supernatants *via* ELISA, whereas NLRP3 protein expression was assessed by Western immunoblot. Data were statistically analyzed by one-way variance analysis and the Fisher least significant difference *post hoc* test. Differences were considered significant when $P \leq 0.05$.

Results: Microscopic examination revealed a typical dendritic morphology of MoDC after differentiation from blood monocytes. The mRNA expression of IL-1β, IL-18, and NLRP3 was increased 6 h and 20 h after bacterial incubation with ETEC ($P \leq 0.05$), but not with *E. faecium*. Caspase-1 mRNA expression was not affected by either bacterial strain. At the mRNA level, the inflammatory response towards ETEC was not prevented by preincubation with *E. faecium*. The protein expression of IL-1β was only enhanced by ETEC treatment 20 h after bacterial addition (36-fold; $P \leq 0.05$). Preincubation with the probiotic tended to reduce this upregulation. At the protein level, NLRP3 was detectable in all treatment groups including the control group.

Conclusion: The results indicate that ETEC is capable of inducing an inflammasome response in porcine MoDC, which could not be prevented by preincubation with the probiotic *E. faecium* strain.

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This research was financially supported by the German Research Foundation (Grant No. Lo 2058/1-1) and by an Elsa Neumann Grant to Henriette Loss.

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94.

The effect of dietary carnitine supplementation and carnitine deficiency on the contractile and metabolic phenotype of skeletal muscle in growing pigs

Wirkung einer diätetischen Carnitinsupplementierung und eines Carnitinmangels auf den kontraktilen und metabolischen Phänotyp des Skelettmuskels wachsender Schweine

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Dietary supplementation of L-carnitine to obese rats was found to improve the carnitine status and to counteract an obesity-induced muscle fiber transition from type I to type II. However, it has not been resolved if the change of muscle fiber distribution and the restoration of the normal muscle fiber distribution in obese rats by supplemental L-carnitine is causally linked with the carnitine status. Thus, it was hypothesized that fiber type distribution in skeletal muscle is dependent on carnitine status. To test this, the carnitine status of young pigs was experimentally manipulated through oral administration of either L-carnitine or sodium pivalate. Pivalate induces carnitine deficiency by forming excessive tissue levels of pivaloylcarnitine esters, which are readily released from tissues and subsequently lost in the urine at a rate that exceeds endogenous carnitine synthesis. Young pigs were used as experimental animals, because the carnitine concentration in skeletal muscle of young pigs can be markedly increased within a relatively short feeding period of about three weeks. **Methods:** The experiment included 48 male crossbred piglets with an age of five weeks and a body weight of 8.22 ± 1.45 kg (mean ± SD, n = 48). The piglets were randomly allocated to four groups of 12 piglets each [control (CON), carnitine (CARN), pivalate (PIV), carnitine + pivalate (CARN+PIV)]. Piglets of all groups received an identical diet, which had a low native carnitine concentration (< 10 mg/kg diet) and met the nutrient requirements according to GfE. In addition, all piglets were given orally either 60 mg sodium bicarbonate/kg body weight (group CON), 20 mg L-carnitine and 60 mg sodium bicarbonate/kg body weight (group CARN), 30 mg pivalate (dissolved in sodium bicarbonate)/kg body weight (group PIV) or 20 mg L-carnitine and 30 mg pivalate/kg body weight (group CARN+PIV) each day for a period of 28 days. The dose of sodium bicarbonate administered to groups CON and CARN was equal to that administered to the groups receiving sodium pivalate. At day 29, the pigs were anesthesized in the post-prandial period by electrical stunning and killed by exsanguination, and plasma, samples from longissimus (L) and superficial biceps femoris (BF) muscles and liver were collected. Carnitine status was assessed by HPLC-MS/MS and muscle fiber typing was performed using myosin heavy chain (MHC) distribution-based immunohistochemistry. In addition, the expression of genes reflecting the metabolic phenotype and genes encoding critical regulators of muscle fiber transition were determined in skeletal muscle. **Results:** Final body weights, daily body weight gain, daily feed intake and feed conversion ratio did not differ between the four groups of piglets. Concentrations of total carnitine in plasma, liver and L and BF muscles were 2.0-2.7 fold higher in group CARN than in group CON, whereas these concentrations were 1.9-2.5-fold lower in group PIV than in group CON ($P < 0.05$). The concentrations of total carnitine in these tissues did not statistically differ between group CARN+PIV and group CON. Fiber type distribution of L and BF muscles, mRNA and protein levels of molecular regulators of fiber distribution in L and BF muscles and mRNA levels of genes reflecting the metabolic phenotype of L and BF muscles did not differ between groups. **Conclusion:** Changes in the systemic carnitine status and the muscle carnitine concentration induced by either supplementing L-carnitine or administering pivalate have no impact on the contractile and metabolic phenotype of skeletal muscles in young pigs.

This study was financially supported by the Deutsche Forschungsgemeinschaft (Grant no. KE 1887/1-1).

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95.

A feeding study on the potential impact of essential oils supplementation on calcium absorption in dairy cows

Eine Fütterungsstudie zum möglichen Einfluss der Supplementierung mit ätherischen Ölen auf die Calcium-Resorption von Milchkühen

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In ruminants, volatile essential oils are among the most commonly used plant bioactive compounds which are commercially available, promoting effects such as improved herd health and increased feed efficiency. In most trials, the use of a blend of essential oils (BEO) as feed supplement is evaluated for its ability to modulate the ruminal microbiota and the resulting changes in fermentational products [1]. However, in recent In vitro studies essential oils were shown to stimulate the ruminal uptake of cations such as Na^{+}, Ca^{2+}, and NH^{4+} across the epithelium of sheep and cattle [2]. These findings indicate that the beneficial effects of essential oils could at least partially be carried out by an interaction with epithelial transport proteins such as transient receptor potential (TRP) channels. Since the absorption of Ca^{2+} plays a particularly important role in the metabolism of lactating cows, this study sought to test whether a BEO supplementation would have an effect on calcium and magnesium uptake and feed efficiency in lactating dairy cows. Methods: In the current study, 72 dairy cows in mid- to end lactation were divided into two groups of 36 animals each and fed the same total mixed ration with or without daily supplementation of 1.2g of a commercial blend of plant bioactive essential oils (BTX12, PerformaNat GmbH, Germany, containing eugenol, menthol and anethole) in a 2x2 cross over design. Blood and urine samples were taken directly after morning milking before starting the BEO supplementation (day 0) and after 21 days of BEO supplementation (day 21); feed intake, milk yield and milk composition were monitored daily. For statistical comparison, the individual changes of parameters between day 0 and day 21were calculated. The resulting value of each animal of the control period was subtracted from the according value of the BEO feeding period. The resulting differences were compared; significance was tested using t-tests. Results: Changes in serum calcium concentration differed significantly between groups: compared to the control group, calcium levels in the BEO group were 0.082 ±0.026 mmol/l higher (p Conclusions: Feeding BEO caused elevated calcium blood levels in dairy cows, suggesting that the mode of action of BEO supplementation might be not solely dependent on its interaction with the ruminal microbiota. The present study supports the hypothesis that enhanced TRP channel mediated cation resorption might contribute to the positive effects of BEO feeding.

The study was funded by the European Social Fund (ESF) and the German Ministry of Economics and Technology based on a decision of the German Parliament (03EUABE057).

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96.

Effects of the probiotic *Enterococcus faecium* NCIMB 10415 on primary cultured porcine adaptive immune cells

Effekte des Probiotikums Enterococcus faecium auf primäre porcine Immunzellen des adaptiven Immunsystems

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Feeding of the lactic acid-producing *Enterococcus faecium* NCIMB 10415 (*E. faecium*), a licensed probiotic for pigs and piglets, has been described to promote growth performance and health in pigs. In a previous study, we demonstrated that *E. faecium* has an immunomodulatory effect in gut associated lymphatic tissues of pigs (1). However, the underlying mechanism of action is still elusive as *E. faecium* may either directly influence immune cells or influence the intestinal milieu. We hypothesize, that *E. faecium* is able to directly act on cytotoxic T-cells and B-cells, two major players of the adaptive immune system. Therefore, we established a porcine *In vitro* cell culture model to explore direct interactions of porcine adaptive immune cells and probiotics.

Methods: We tested the direct effects of *E. faecium* on adaptive immune cells in *In vitro* co-culture experiments of peripheral blood mononuclear cells (PBMCs) of German Landrace pigs and vital or UV-inactivated *E. faecium*. For validation, immune cells were repeatedly isolated from blood of up to six independent donor pigs sharing the same genetic and environmental background. In addition, we analyzed immune cells isolated from mesenteric lymph nodes from adult slaughter pigs. For experiments, either 1 x 10^6 PBMCs or 0.5-1 x 10^6 of magnetically separated B-cells were treated with vital or UV-inactivated bacteria (killed via UV-light radiation for 3 hours) from the probiotic strain *Enterococcus faecium* NCIMB 10415 (Cylactin, Cerbios Pharma) in a ratio of 1:2, 2:1, or 10:1 (PBMCs : *E. faecium*) for 1, 3, 5 or 20 hours in RPMI 1640 without glucose. After that, cells were harvested on ice and stained with T and B-cell specific antibodies and analyzed via flow cytometry (Canto, BD). The relative cell count was calculated as the percentage of live lymphocytes. Dead-live discrimination was assessed using DAPI-staining. Otherwise, cells were stored at -80 °C to perform RNA extraction and qPCR of immune relevant genes. Data were analysed using R version 3.2.3. Effects of the *E. faecium* treatment against controls without bacteria were tested using pairwise Mann-Whitney-U-Tests. The result was considered significant when $p < 0.05$.

Results: We detected higher relative cell counts of CD8b+ cytotoxic T-cells ($p < 0.05$) in the treatment group with vital *E. faecium* after 1, 5 and 20 hours of incubation compared to untreated controls within PBMCs. In a separate experiment, in which we treated primary cultured lymphocytes derived from mesenteric lymph nodes from slaughter pigs with *E. faecium*, we co-stained CD8b+ cells with the activation marker CD27. If CD27 is lost, T-cells had antigen contact and were therefore activated (2). We found a tendency towards higher relative cell counts of CD8+CD27- cytotoxic T-cells ($p<0.1$) for lymphocytes treated with vital *E. faecium* bacteria compared to the control with no *E. faecium* treatment. Treatment with UV-inactivated bacteria did not result in changes. By analyzing another important adaptive immune cell type, the B-cells, we observed a different pattern. We found higher relative cell counts of CD21+ B-cells ($p<0.05$) and correspondingly higher relative cell counts of CD79+ B-cells ($p<0.1$) in treatments with UV-inactivated *E. faecium*, while there was no effect with vital *E. faecium*, but rather a trend towards lower relative cell counts. In addition, we measured a trend towards lower expression of B-cell regulatory (IGLC, IGKC) and activation marker genes (CD40, CD2) in treatments with vital *E. faecium* on magnetically sorted CD21+ B-cells.

Conclusion: We were able to find hints of a stimulation of cytotoxic T-cells through vital *E. faecium*. These results suggest that cytotoxic T-cells need a vital bacterium and were potentially stimulated by secreted factors of *E. faecium*. We suggest a specific immunomodulatory effect of *E. faecium*, which presumable influences the direction of immune response towards an enhanced cytotoxic T-cell answer at expense of the B-cell response. This study could provide evidence of a direct immunomodulatory effect of *Enterococcus faecium* NCIMB 10415 on adaptive immune cells *In vitro*.

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97.

Gradual replacement of dietary maize grain with molassed sugar beet pulp modulates ruminal and hindgut fermentation profile in high yielding dairy cows

Ersatz von Mais durch melassierte Trockenschnitzel moduliert die Fermentation in Pansen und Dickdarm hochleistender Milchkühe

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Introduction: High starch diets can cause major problems in dairy production systems such as subacute ruminal acidosis, hindgut acidosis and milk fat depression. Replacing grain with sugar beet pulp (Bp) is a viable method to reduce the dietary starch content and to elevate ruminal pH by modulating the ruminal fermentation profile in favour of acetate. However, little is known about the effect of increased dietary Bp levels on hindgut fermentation and the faecal pH. Thus, the present study evaluated the effects of a gradual replacement of maize grain with Bp on ruminal and hindgut fermentation.

Material and methods: Three diets with increasing amounts of Bp were tested using 18 high yielding (36.5 ± 0.5 kg/d), early lactating (37.4 ± 0.9 days in milk) Simmental cows (745 ± 199 kg). Dietary Bp inclusions were 0% (CON), 12% (12Bp) and 24% (24Bp), thereby replacing respective amounts of maize grain (dry matter basis). To allow the measurement of baseline values all cows were fed the CON diet for 7 days. Afterwards they were assigned to one of the three diets, which were fed for 21 days. Ruminal pH was measured continuously using wireless pH sensors (smaXtec premium, smaXtec animal care GmbH, Graz, Austria). Ruminating and chewing behaviour was measured on the last 4 days of the experiment, using noseband sensors (Rumiwatch, ITIN & HOCH GmbH, Fütterungstechnik, Liestal, Switzerland). Faecal grab samples were taken on the last day of the experiment before the morning milking and were analyzed for short chain fatty acids (SCFA) using gas chromatography and for pH using a pH-meter (SevenGo, Mettler Toledo, Columbus, OH, USA).

Results: Replacing dietary maize grain with Bp did not affect mean ruminal pH ($P = 0.894$) that averaged 6.25 over all experimental groups. In addition no effect was found on the daily pH maximum ($P = 0.140$) and the time below pH 6.0 ($P = 0.270$). However, the daily pH minimum was increased from 5.8 to 6.0 ($P = 0.049$) and the daily pH range (max – min) was decreased from 0.77 to 0.58 ($P < 0.001$) with the 24Bp diet compared to CON. No diet effect was found on the chewing and ruminating activity ($P > 0.05$). The faecal pH tended to increase from 7.42 to 7.67 ($P = 0.087$) with the 24Bp diet, while no diet effect on total SCFA was found (on average 39 mmol/l). However, dietary inclusion of Bp tended to quadratically affect the relative faecal acetate proportion of total SCFA ($P = 0.071$) that was lowest with the 12Bp diet. No effect was found on the faecal propionate ($P = 0.703$). But the relative faecal butyrate concentration decreased linearly with dietary Bp inclusion from 10.0% with CON to 7.3% with 24Bp ($P < 0.001$).

Conclusion: Results showed that the dietary replacement of maize grain with Bp decreases the risk of subacute ruminal acidosis by elevating the daily ruminal pH minimum and by reducing the daily pH fluctuation. In addition, feeding the 24Bp diet slightly elevated faecal pH indicating less hindgut fermentation, likely due to the reduction of dietary starch. Only small effects were found on the hindgut SCFA pattern, indicating that dietary Bp inclusion is not suitable to promote acetate production in the hindgut.

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98.

Does fishmeal substitution by partly defatted insect meal (*Hermetia illucens*) or microalgae powder (*Arthrospira platensis*) impact on feed acceptance and growth of rainbow trout?

Beeinflusst die Fischmehlsubstitution durch teilentfettetes Insektenmehl (Hermetia illucens) oder Mikroalgenpulver (Arthrospira platensis) die Futterakzeptanz und das Wachstum von Regenbogenforellen?

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The rapid development of aquaculture has intensified the need for a diversification of nutritionally appropriate aquafeed ingredients. Both insect and microalgae meals have the nutritional potential to reduce the dependence on conventional protein sources in aquafeed. The aim of the current study with Rainbow trout was to evaluate feed acceptance and growth performance following substitution of fishmeal (FM) by partly defatted insect meal (*Hermetia illucens*) or microalgae powder (*Arthrospira platensis*).

Methods: A growth trial was conducted with 600 juvenile Rainbow trout (*Oncorhynchus mykiss*) using a control diet based on 20% FM, 20% soy protein concentrate (SPC) and four experimental diets with 50 and 100% substitution of FM by spray-dried Spirulina powder (SP; Crude protein (CP) = 69% of dry matter (DM); diets SP10, SP20) or partly defatted *Hermetia* meal (HM; CP = 61% of DM; Crude lipids = 14% of DM; diets HM10, HM20). In addition, a diet with complete replacement of both FM and SPC by 40% of insect meal was created (HM40). Diets were formulated to be similar both in CP (47% of DM) and digestible energy content. Lys and Met supplementation ensured indispensable amino acid supply as recommended (1). Feed intake and growth response were investigated over 27 days making use of a semi-closed in-door water recirculation system with 30 tanks (320 l/tank; water temperature 16.9 ± 0.3°C; regulated photoperiod 14h light/10h dark) and five replicate tanks per diet (20 fish per tank) with feed supply on free choice level by hand (two meals per day). Response parameters were calculated according to (2). Statistical analyses (one-way ANOVA, Tukey-test) utilized the SPSS-software (IBM SPSS Statistics 24.0).

Results: All diets were very well accepted. Replacement of FM by SP or HM increased feed intake (significantly with diets SP10 and HM10). Growth data (WG, SGR) were similar between diets. HM replaced FM completely without negative effects both on feed conversion ratio (FCR) and protein efficiency ratio (PER). However, 50% of FM can be substituted successfully by SP. Elevation of the dietary HM content by complete replacing of SPC tended to impair both FCR and PER (Table). Furthermore, the dietary inclusion of SP yielded a yellowish colour of fillets.

Diet	Control	SP10	SP20	HM10	HM20	HM40
FI [%BW/d]	$1.9^{a} \pm 0.2$	$2.3^{b} \pm 0.1$	$2.2^{ab} \pm 0.1$	$2.2^{b} \pm 0.2$	$2.1^{ab} \pm 0.1$	$2.1^{ab} \pm 0.8$
WG [g]	47.8 ± 11.9	55.9 ± 8.7	45.2 ± 6.1	52.1 ± 6.1	49.3 ± 4.0	48.1 ± 2.6
SGR [%/d]	2.9 ± 0.5	3.3 ± 0.2	2.9 ± 0.4	3.2 ± 0.3	3.1 ± 0.3	2.9 ± 0.1
FCR [g/g]	$0.85^{a} \pm 0.08$	$0.87^{ab} \pm 0.02$	$0.96^{b} \pm 0.07$	$0.87^{ab} \pm 0.04$	$0.89^{ab} \pm 0.05$	$0.93^{ab} \pm 0.03$
PER [g/g]	$2.52^{a} \pm 0.23$	$2.45^{ab} \pm 0.05$	$2.21^{b} \pm 0.17$	$2.47^{ab} \pm 0.12$	$2.46^{ab} \pm 0.14$	$2.29^{ab} \pm 0.08$

Means (± SD); FI = feed intake; WG = weight gain; SGR = specific growth rate; FCR = feed conversion ratio; PER = protein efficiency ratio; different superscript letters reveal significant differences between diets ($p<0.05$)

Conclusion: Both alternative protein sources under study can be used to replace FM in diets for Rainbow trout but results indicate that partly defatted insect meal (*Hermetia illucens*) is more suitable to substitute FM (up to 100%) than Spirulina (*Arthrospira platensis*) powder (only 50%). Impact on fillet colour when applying SP should be considered since it can affect consumer acceptance. Additional elevation of dietary HM is not recommended as it tended to decrease growth performance. Ongoing research will focus on protein utilization for further improvement of the dietary amino acid supply in trout diets by alternative protein sources.

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99.

Effect of soaking and steaming hay on the consumption rate and chewing activity in horses

Einfluss des Wässern und Dämpfens von Heu auf Futteraufnahmedauer und Kauaktivität bei Pferden

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Haymaking is often complicated by poor drying conditions resulting in a decreased hygienic quality with elevated dust release from abiotic and biotic contaminants. Soaking and steaming might counteract the latter, which is important particularly for horses with respiratory problems (1). However, soaking for 15 minutes leaches also important nutrients (e.g. soluble protein, amino acids [AA]) as well as water soluble carbohydrates (WSC) where the latter is advantageous for horses with disturbed insulin sensitivity (2). In general, treatment induced changes in dry matter (dm) content and texture/ grip may influence ingestion behavior (3), which needs to be characterized. The aim was to investigate how soaking and steaming of hay, respectively, influences consumption rate and chewing activity of horses. We hypothesized that both treatments have an impact of the issue in question.

Methods: 6 adult warmblood mares (bwt 536 ± 36.0 kg) were fed 2 meals/d hay (one batch, first cut, end of blossom, ME = 0.52 MJ ME/kg bwt0.75/d) in 3 treatments according to a cross-over design: native (NAT), soaked (SOA, 15 min soaking, 10 min dripped), steamed (STE, HAYGAIN, 90°C 1h) for 5d adaptation period. On d6 and d7 the horses were equipped with modified halters and got 2 kg of the treated hay for 1h and chewing parameters were detected (feed intake time, FIT in min/kg dm; consumption rate, CR in g dm/min; chewing frequency, CF in chewing cycle [CC]/sec); chewing intensity, CI in CC/kg dm). Statistical analysis for chewing parameters was conducted with SAS 2.4.

Results: Reduction of WSC contents was more pronounced in SOA, whereas fiber fractions increased in parallel. Both treatments reduced crude protein (CP), but STE led to a higher decrease of precaecal digestible (pcd) CP (tab). This also applies to pcdAA ([g/16g N], pcdLys; NAT, 1.91; SOA, 1.79; STE, 1.31; pcdMet+Cys, NAT, 1.14; SOA, 0.96; STE, 0.73; pcdThre, NAT, 1.84; SOA, 1.75; STE, 1.32). SOA *vs* NAT and STE caused higher CI ($P<0.05$), a higher CF ($P=0.08$) and a lower CR ($P<0.05$). Observations suggest decreased acceptance if horses were fed SOA, but not NAT or STE.

hay	*dm*	*CP*	*pcdCP*	*CL*	*WSC*	*aNDFom*	*ADFom*	*CF*	*CF30*	*CI*	*CR*
	[g/kg]					*[g/kg dm]*		*[CC/sec]*		*[CC/kg dm]*	*[g dm/min]*
NAT	929	94	55	13	9.4	625	366	1.05	1.11	2622b	24.4a
SOA	307	73	42	7	6.9	706	471	1.091	1.13	3537a	19.5b
STE	681	70	33	6	8.4	702	465	0.901	1.05	2521b	21.5a

1$P=0.08$, ab$P<0.05$ within a column; CP, crude protein; CL, crude lipids; WSC, water soluble carbohydrates; CF, chewing frequency; CC, chewing cycle; CI, chewing intensity; CF30, first 30min of feed intake; CR, consumption rate

Conclusion: Soaked and steamed hay differs considerably from its native counterparts in term of dry matter and nutrient contents and texture/grip as well. Particularly the decrease in the pcd part of CP and essential AA in the steamed hay needs further investigation. Soaking induced a higher CF and CI combined with a reduced consumption rate but also the most effective reduction in WSC, which actually should be rated beneficial especially for horses with disturbed insulin sensitivity. Maybe the profitable chewing parameters for soaked hay can also be an expression of decreased acceptance. Consequences for the energy and nutrient intakes should be payed attention.

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100.

Relationship between particle size distribution (PSD) and nutrient composition in samples of mash diets for laying hens and its potential diagnostic value for feed consulting

Beziehung zwischen der Partikelgrößenverteilung und der Nährstoffzusammensetzung in Proben schrotförmiger Mischfutter für Legehennen und dessen möglicher diagnostischer Wert für die Futterberatung

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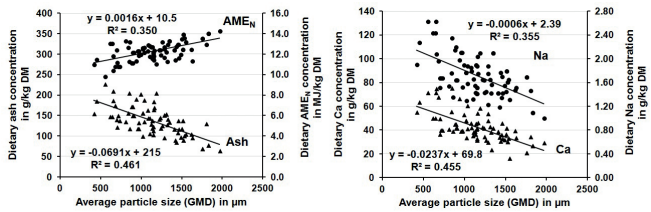
Diets for laying hens have to be designed with a nutrient composition and feed structure ensuring a sufficient supply of hens with energy and nutrients as well as a chicken-specific feeding behaviour. For this reason, mash diets are used in hen feeding mainly. However, this type of diet has the disadvantage of being prone to undesirable deviations in PSD caused by excessive or insufficient grinding of feed components. Intensifying the separation of fine and coarse feed components increasing heterogeneity in diet particles can impede feed intake and sufficient nutrient supply of hens affecting their performance and animal health adversely. In this context, this study examined the relationship between PSD and nutrient composition in samples of mash diets for its potential diagnostic value in determining nutrition-associated performance and health issues in hen flocks.

Methods: The present study included a meta-analysis of data on energy and nutrient concentrations as well as PSD of 82 mash diets (complete feeds) for laying hens from commercial farms that were examined at our institute from 2015 to 2017. Based on the flocks" case history, diets were classified into the following five groups: No symptoms (A; n=19), decline in laying performance (B; n=25), decline in hens" live body weight (C; n=10), feather pecking (D; n=12) and cannibalism (E; n=16). In accordance with VDLUFA methods feed samples were analysed for dry matter, crude nutrients, starch, sucrose, sodium (Na), calcium (Ca) and phosphorous. Afterwards, the dietary concentration of metabolisable energy (AMEN) was calculated [1]. In accordance with the industrial norms DIN 66165-1 and DIN 66165-2 the PSD was examined in all samples via dry sieving analysis using test sieve shaker HAVER EML 200 Premium Remote® (Fa. Haver & Boecker) equipped with 8 sieves of 3150, 2000, 1400, 1000, 800, 500, 400 and 200 µm sieve diameter according to DIN ISO 3310-1. Each sample was sieved for 10 minutes with an amplitude of 2.0 mm. Thereafter, sieves were weighed and particle proportions in samples as well as their geometric mean diameter (GMD; average particle size) were calculated according to [2]. For statistical evaluation of data a one-factorial ANOVA with „case history" as fixed group effect was performed using SPSS 22.0 procedure ANOVA (2013) with Student-Newman-Keuls test. Differences between groups were considered as significant for $p \leq 0.05$. In addition, using linear regression analysis (SPSS 22.0, 2013) relationships between nutrient concentrations and GMD in feed samples were determined independently of samples" case history.

Results: Mash diets from groups D and E showed lower proportions of fine (200 to 800 µm) and higher proportions of coarse particles (>1400 µm; mainly whole and insufficiently ground grains and protein providers) and were more inhomogeneous than those from groups B and C ($p<0.05$). Comparably, the GMD trended to be lower in B and C diets than in D and E diets (1050±334 µm vs. 1314±300 µm; $p=0.06$). Independent of samples" case history, the linear regression analysis revealed that the higher the GMD of mash diets the higher their AMEN and the lower their mineral concentrations (ash, Ca and Na; see figure) are. Accordingly, the coarser D and E diets showed significantly higher starch and AMEN concentrations than the finer B and C diets ($p<0.05$).



Conclusions: The present study revealed that in samples of mash diets GMD variations are moderately correlated with shifts in dietary mineral and energy concentrations. Serving as potential risk factors for feather pecking and cannibalism in hen flocks, increasing coarseness and heterogeneity of particles promote separation processes between mineral (fine) and starch containing (coarse) feed components. Regarding the differences in GMD and nutrient composition between samples with different case history, PSD analysis in mash diets might be a valuable tool for the determination of nutrition-associated performance and health issues in hen flocks.

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101.

Influence of „controlled fermentation“ on D- and L-lactic acid and volatile fatty acid contents in a cereal- and rapeseed meal-based liquid diet for pigs

Einfluss der Kontrollierten Fermentation auf den Gehalt an D- und L-Milchsäure sowie an flüchtigen Fettsäuren in einem Flüssigfutter für Schweine auf der Basis von Getreide und Rapsextraktionsschrot

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Controlled fermentation is an increasingly popular liquid feeding concept in pig production. In practice, fermentation has been proven to be successful in a „batch-process“. The 35-40 °C warm liquid feed is prepared daily with a certain amount of starter culture added at the beginning of fermentation. The liquid feed is stored under semi-anaerobic conditions for at least 24 h before feeding. The aim of fermentation is a lowering of the pH value of the diet by high lactic acid content and low levels of volatile fatty acids (VFA). Fermented liquid feed (FLF) promises to improve phosphorus and protein digestibility and influences the intestinal flora of pigs positively [1]. The aim of the experimental study was to compare lactate and VFA levels in a fermented and a native liquid diet to evaluate potential effects on palatability and gastrointestinal health.

Material and Methods: The preparation of the FLF was carried out in two identical mini-fermenters (Mini-Fermenter 125 ltr., WEDA Dammann & Westerkamp GmbH, Germany). The mini-fermenters were filled daily at 7.30 am and the feed was fermented for 24 h. The mini-fermenter was closed after filling and the liquid feed was stirred therein every hour for 60 seconds at 900 rotations. Equipped with a heating coil, a fermentation temperature of 35-38 °C was ensured over the entire fermentation period. The fermenters were thoroughly cleaned after fermentation and refilled the next day. The diet consisted of 50% rye, 30% rapeseed meal, 10% wheat, 10% barley and was mixed in a feed:water ratio of 1:3.2. Without adding starter culture pH value was not lower than 4.0 after 24 h of „uncontrolled fermentation“. Therefore a freeze-dried, granulated starter culture (SCHAUMALAC FEED PROTECT XP G, H. Wilhelm Schaumann GmbH, Germany) was added at the beginning of fermentation in a dosage of 2 x 10^5 cfu/g liquid diet. Immediately before the starter culture was added, pH value was measured and the content of volatile fatty acids was determined gas chromatographically (610 Series, Unicam, Germany) and D- and L-lactate were measured enzymatically (D-Lactic acid/L-Lactic acid UV Test, Boehringer Mannheim, Germany). After 24 h of fermentation pH value, volatile fatty acid contents and D- and L-lactate contents were determined again.

Results: The following table presents effects of controlled fermentation (n=3; Ø ± SD):

	pH	D-lactate[mmol/kg DM]	L-lactate[mmol/kg DM]	Acetic acid[mmol/kg DM]	Butyric acid[mmol/kg DM]
Before fermentation	5.95± 0.025	1.27± 0.213	1.68± 0.550	11.7± 0.783	0.067± 0.052
After 24 h of fermentation	3.72± 0.058	314± 44.0	349± 30.4	122± 24.8	0.210± 0.057

The controlled fermentation of the cereal and rapeseed meal-based diet led to lower feed pH values reaching less than 4.0 within 7-8 h. In addition to high D- and L- lactate levels, small increases in acetic acid and butyric acid were measured.

Conclusion: FLF provides high levels of lactate, which are nearly 10 times higher than the stomach lactate content in conventional pig feeding [2]. That might influence the pH value in the digesta of the small intestine also. Acetic acid content was less than 166 mmol/kg DM in every FLF sample, so that this content probably will not influence feed palatability in pigs negatively [3]. With the mini-fermenters, the target values (pH,VFA, lactate) from the large-scale fermenters established in practice could thus be achieved.

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102.

Does the substitution of soy protein by novel protein from microalgae *Arthrospira platensis* impair the dietary protein quality of Tilapia feed?

Hat die Substitution von Sojaprotein durch neuartiges Mikroalgenprotein aus Arthrospira platensis eine Verschlechterung der Proteinqualität von Tilapiafutter zur Folge?

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Up to now, both fishmeal (FM) and soy bean products are important protein sources in aquaculture nutrition. Due to rapid development of aquaculture industry and the increasing need for sustainable aquafeeds nutritional research has been focused on alternative protein sources. Microalgae have the potential to reduce the dependency on traditional protein sources in aquafeed (1). The current study aimed to evaluate protein quality parameters of Tilapia diets with substitution of soybean protein by the microalgae Spirulina (*Arthrospira platensis*).

Methods: A growth study utilized 400 juvenile all male Nile tilapia (*O. niloticus*). A control diet (7% FM, 31% soy protein concentrate (SPC; Crude protein (CP): 74% of dry matter (DM)) and four experimental diets with 50, 75 or 100% replacement of SPC by spray-dried Spirulina powder (SP; CP: 69% of DM) were examined. Diets (SP50, SP75, SP100) were formulated to be similar both in CP (38.5% of DM) and energy content. Essential amino acid supply of the diets was within the recommendations for Tilapia, except diet SP100. The observed Lys deficiency was eliminated by additional Lys supplementation (diet SP100+Lys). Growth response and protein utilization were studied in a semi-closed in-door water recirculation system with 20 tanks (320 l/tank; water temperature 24.7 ± 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 feeding two meals per day at DM based feed supply of 2.2% of 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 (NPU_{std}) referred to an average of daily N intake (NI=430mg/$BWkg^{0.67}$) 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 SP significantly improved protein quality (NPU_{std}), specific growth rate (SGR) and feed conversion ratio (FCR) (Table). Parameters tended to decline when the SPC replacement level exceeded 75%. Supplementing Lys improved NPUstd significantly, indicating that Lys became the limiting amino acid in diet SP100.

Diet	Control	SP50	SP75	SP100	SP100+Lys
NPU_{std} [%]	$43.2^{a} \pm 0.8$	$47.3^{b} \pm 1.0$	$49.8^{cd} \pm 1.4$	$47.9b^{c} \pm 1.1$	$50.6^{d} \pm 0.8$
SGR [%]	$4.1^{a} \pm 0.1$	$4.7^{b} \pm 0.1$	$5.0^{b} \pm 0.2$	$4.8^{b} \pm 0.1$	$4.9^{b} \pm 0.2$
FCR [g/g]	$1.37^{a} \pm 0.04$	$1.19^{b} \pm 0.04$	$1.10^{c} \pm 0.05$	$1.14^{bc} \pm 0.04$	$1.12^{bc} \pm 0.03$

Means (± SD); SGR = specific growth rate; FCR = feed conversion ratio; NPU_{std} = standardized net protein utilization (standardized daily N-intake = 430mg/$BWkg^{0.67}$); different superscript letters reveal significant differences between diets ($p<0.05$)

Conclusion: Replacement of SPC by spray-dried Spirulina powder improved dietary protein quality of Tilapia diets under study and enhanced growth performance of Nile tilapia. If replacement of SPC exceed 75% (23% Spirulina meal in the diet), Lys supply became the limiting factor. It has been shown that Spirulina (*Arthrospira platensis*) is a promising alternative to common protein sources on the road to a more sustainable mixed feed for Tilapia.

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103.

The use of condensed tannins as silage additive for rehydrated corn grain: effects on silage fermentation pattern and losses

Verwendung von kondensierten Tanninen als Siliermittel für Maiskörner: Auswirkungen auf Gärungsmuster und Verluste der Silage

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Ensiling is a method used to conserve herbage or grains through acidification by microbial fermentation. However, the development of spoilage microorganisms (as *Clostridium* spp. and fungi) during storage phase reduces the conservation efficiency and increases the nutrient losses. Controlling the fermentation by using tannins as additive could reduce the risk of undesirable fermentation. We aimed to evaluate the effect of a quebracho tannin extract (QTE) as silage additive on fermentative losses and fermentation pattern of rehydrated corn grain silage.
Methods: For ensiling, corn grain (*Zea mays*) was utilized. After grinding (5mm) and rehydration (35% of moisture), rehydrated corn was inoculated with a bacterial inoculant (Bon Silage) containing 1k2075 *Lactobacillus buchneri*, 1k20711 *Lactobacillus ramnosus,* and 1k2079 *Lactobacillus plantarum,* in order to reach a theoretical rate of 2.5×10^{11} CFU g^{-1} of silage (as fed). The Quebracho tannin extract was utilized (derived from *Schinopsis lorentzii*), containing 108.47 g kg^{-1} DM of condensed tannins and protein binding capacity (ED50) of 1.18 mg (amount of extract needed to precipitate 50% of test protein in solution). Quebracho tannin extract was added in five different dosages: 0 (control), 5, 10, 30 and 50 g kg^{-1} DM of silage. The experimental silos were vacuum-sealed bags (Komet plus Vac 20), remaining stored for 75 days, and four silos (3 kg each) were produced per treatment. A sample of 150 g of silage (as fed) was utilized to access the dry matter at 55°C (DM). The pH was evaluated in the silage/water extract (9 g of silage as fed in 60 ml of distilled water). Dry matter recovery index (DMRI) and gaseous loss (GL) were determined. For fermentative pattern evaluation, lactic acid (1), biogenic amines (2) and volatile fatty acids (VFA) were measured (3). Data were analyzed using the REG procedure of SAS. For the quadratic equations, the critical point coordinates of $f(\beta, x)$, respectively, $x_{critical}$ and $y_{critical}$, were obtained by $-(\beta_1 \div 2\beta_2)$ and $-[(\beta_1^2 - 4\beta_2) \div \beta_0 \times 2\beta_2]$.
Results: Treated silages showed higher DM content, as well as the DMRI, however, the GL were lower (Table 1). For pH, a quadratic response was observed and the highest point of 4.2 was found using 37.7 g kg^{-1} DM of QTE. However, QTE addition has not caused any impairment for the lactic acid synthesis in this study. Nevertheless, the VFA production was altered by the QTE addition, once reduced contents of butyric acid and acetic acid were found. Propionic acid content was not affected. Treated silages had a lower content of biogenic amines. Quebracho tannin extract addition in silages linearly decreased the concentration of methylamine, putrescine, cadaverine, and spermidine. For spermine, the minimum point of 6 mg kg^{-1} DM was observed using a dose of 28.2 g kg^{-1} DM of tannin extract.
Table 1: Effect of quebracho tannin extract addition (g kg^{-1} DM) on dry matter, pH, fermentative losses, and fermentation pattern of rehydrated corn silage.

Variable	Regression Equation	R^2	$x_{critical}$	$y_{critical}$
DM[a]	64.84+0.02174X	0.66		
pH	3.967+0.01155X-0.0001532X^2	0.85	37.7	4.2
DMRI[b]	63.3+0.0312X	0.35		
GL[c]	2.28-0.025X	0.93		
Acetic Acid[d]	0.628-0.00968X	0.78		
Iso-Butyric Acid[d]	0.906-0.0167X	0.67		
Methylamine[e]	41.45-0.986X	0.71		
Putrescine[e]	29-0.39X	0.95		
Cadaverine[e]	146.03-2.97X	0.82		
Spermidine[e]	24.21-0.34X	0.57		
Spermine[e]	13.68-0.546X+0.00967X^2	0.92	28.2	6

[a] g kg^{-1} as fed; [b] % of DM; [c] % of initial DM; [d] g kg^{-1} DM; [e] mg kg^{-1} DM; [f] Critical value of coordinates x (g kg^{-1} DM) and y.
Conclusion: Quebracho condensed tannin extract usage reduced fermentative losses and undesirable fermentation without impairing silage conservation.

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The study was supported by CAPES Foundation (Brazil).

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104.

Influence of different sources and length of fibers in the diet on the growth of broiler chickens

Einfluss der Herkunft und der Länge der Faser im Futter auf das Wachstum von Mastbroilern

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The dietary fibers from all feedstuffs in diets of poultry have different task related to health and welfare topics. Neutral Detergent Fiber (NDF) measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose). It is recommended to use 35 g crude fibers per kg in broiler starter feed and with older broilers 40-45 g/kg (1). Information on recommendations for an optimal length of the fiber in broiler feed cannot be found. Therefore, the following study was carried out to investigate the influence of different sources and lengths of fibers in the diet on the growth performance of broiler chickens.

Methods: 630 one-day old male chickens (ROSS) were randomly distributed into six treatment groups (two fiber origins – wheat/oat, 3 fiber lengths (SANACEL® – 30/200/400 or 300 µm) and one control group with 9 pens per group over a study period of 42 days (Table 1). 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. Data were analyzed via a two-way ANOVA (SAS).

Table 1: Main ingredients and analyzed nutrients of the diets (g/kg)

Feed stuffs /Groups	Control -1	Wheat-fiber - 2/3/4	Oat-fiber - 5/6/7
Corn +wheatDried grass mealFiber – wheat, 30/200/400 µmFiber – oat, 30/200/300 µm	48844--	4883410-	48834-10
Crude protein (CP)Crude fiber/NDFE, MJ/kg	21540/11512.80	21441/12012.80	21543/13112.80

Results: The results of this study indicate that an inclusion rate of different fiber lengths not significantly influenced feed intake, final body weight, carcass yield and proportional content of gizzard. However, the fiber length of 300/400 µm decreased the feed to gain ratio significantly (Table 2).

Table 2: Performance parameters of broilers; d1 - d42 (means)

G/Fiber /F. length	Feed intakeg/d	Final body weight, g	Feed to gain ratio, g/g	Carcass%	Gizzard%
1/Grass meal/-	110.5	3378	1.392	74.9	0.98
2/Wheat/30	110.7	3377	1.395	74.4	0.87
3/Wheat/200	110.5	3346	1.405	75.3	0.81
4/Wheat/400	108.3	3356	1.375	75.1	0.91
5/Oat/30	109.4	3328	1.398	74.4	0.94
6/Oat/200	110.9	3403	1.387	74.9	0.96
7/Oat/300	110.2	3454	1.358	74.4	0.91
Anova (p-values)					
Fiber origin	0.800	0.400	0.300	0.200	0.200
Fiber length	0.700	0.600	*0.020*	0.200	0.900
F. origin x F. length	0.600	0.400	0.600	0.700	0.400

Conclusion: The results showed that the supplementation of 10 g fiber per kg feed with a fiber length of 300/400 µm and with a total fiber concentration of approximately 40 g/kg feed proved to be especially favorable for the feed to gain ration of the broiler chickens.

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105.

Comparison of the fermentation traits of the barley mutant *rob1* and the wildtype barley cv. Optic in an *In vitro* incubation study using the Rusitec system

Vergleich der Fermentationsparameter von der Gerstenmutante rob1 mit der Ausgangssorte Optic in einem In vitro Inkubationsversuch mit Rusitec

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Barley (*Hordeum vulgare L.*) is the fourth most important cereal crop in the world after wheat, maize, and rice. Because of its high lignin content barley´s use as whole plant forage for ruminants is limited. However, the genetic manipulation of lignin genes can lead to structural and compositional changes in lignin affecting its digestibility. Hitherto a commercially unexploited mutant of barley is the orange lemma (*rob1*) trait which is associated with a lower lignin content. Thus, the aim of the present study was to compare the *In vitro* nutrient degradability and fermentation parameters of the whole plant barley mutant *rob1* in comparison with the wildtype cv. Optic in the incubation system Rusitec at two different stages of harvest.

Methods: Two-rowed spring barley cv. Optic and the EMS induced orange lemma (*rob1*) mutant line in Optic (1), were grown in 2016. For each variety three replications were sown. At booting (73 days after sowing) and milk dough (90 days after sowing) plants from an area of 1 m² of each replicate were harvested and air-dried. Plants were incubated in the Rusitec system in two experimental runs (each lasting 10 days) resulting in 6 replicates per plant and harvest time. The last 5 days of each incubation run were used for daily sampling the fermenter fluid to measure gas production, concentration of short chain fatty acids (SCFA), NH_3, and the nutrient degradation of the plants after 48 h of incubation. Mean values were subjected to the mixed procedure of SAS (9.4, SAS institute inc., Cary, NC) with barley plant, time of harvest, and their interaction as fixed effects.

Results: Acid detergent lignin (ADL) contents were higher at milk dough than at booting. The mutant *rob1* had lower ADL contents than Optic, both at booting (1.39% vs. 1.85% in DM) and at milk dough (2.60% vs. 2.86% in DM). Nutrient degradation differed between the two harvest times, showing a decreased degradability of organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) when plants were harvested at milk dough.

	Optic		rob1			*P*-value		
Degradability (%)	booting	milk dough	booting	milk dough	SEM	Barley type	Time of harvest	Interaction
DM	65.8^{a}	60.5^{b}	67.8^{a}	56.7^{c}	1.07	0.267	<0.001	0.008
OM	64.7^{ab}	61.0^{b}	66.6^{a}	55.4^{c}	1.07	0.103	<0.001	0.003
CP	75.6	51.5	74.3	57.8	3.49	0.234	<0.001	0.081
NDF	53.7^{b}	42.8^{c}	61.7^{a}	39.6^{c}	2.07	0.242	<0.001	0.011
ADF	51.6	38.7	59.0	39.2	2.14	0.079	<0.001	0.123
ADL	23.2	27.2	16.7	25.8	9.96	0.272	0.074	0.479

In this respect negative correlations were found (P<0.001) between ADL content and degradation of OM (r= -0.698), CP (r= -0.840), NDF (r=-0.853) and ADF (r=-0.866). Degradability of ADL was in tendency higher in barley plants harvested at milk dough (P=0.074). Due to the lower degradability of plants harvested at milk dough, the concentrations of SCFA and NH_3 were lower (P<0.05), but overall concentration of NH_3 was higher with *rob1* compared to Optic (P<0.001). Methane formation (ml/day) was on average 22% higher when Optic was incubated compared to *rob1* (P<0.05), with an in tendency higher amount produced when barley harvested at milk dough was used (P=0.055).

Conclusions: Analysis of the fiber fractions confirmed that *rob1* had lower ADL contents than Optic and NDF and ADF degradability was higher when *rob1* was incubated compared to Optic, but only when the plants were harvested at booting. At the later harvest time NDF content was higher in *rob1* and nutrient degradability was decreased.

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106.

Effects of a ration change from a Total Mixed Ration to Pasture combined with concentrate supplementation on metabolism of Dairy cows

Einfluss eines Rationswechsels von einer totalen Mischration auf eine weidebasierte Fütterung mit Kraftfuttersupplementierung auf den Stoffwechsel von Milchkühen

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The transition from a total mixed ration (TMR) to pasture in spring requires metabolic adaptations. It was shown that transition from TMR to pasture combined with a low concentrate supplementation strategy (1.75 kg/cow/day (d), dry matter basis (**DM**)) caused an energy deficit and lipomobilization (Schären et al., 2016). Therefore, the aim of the present study was to examine whether a higher concentrate feed supply (4.5 kg/cow/d (DM)) and a different grazing system would prevent an energy deficit as evaluated by changes of body fat depots and liver fat content.

Methods: In 2016, another pasture trial, approved by the legal authority, was performed with 43 German Holstein cows. The animals (week (wk) 24 of lactation and 30 kg milk/cow/d; parity: 2.2 ± 1.4; mean ± SD) were divided into a pasture- (**PG**, n=22) and confinement group (**CG**, n=21). The CG received a TMR (35% maize silage, 35% grass silage, 30% concentrate, DM-basis) during the whole experiment, whereas the PG was transitioned to pasture wk 0 and 1: TMR-only, wk 2: 3 h/day on pasture, wk 3 and 4: 12 h/day on pasture, wk 5 to 11: pasture-only plus concentrate). Individual milk yield, body condition score (BCS) and bodyweight (BW), blood samples (serum non-esterified fatty acids [NEFA] and beta-hydroxybutyrate [BHB] concentrations) were taken weekly and milk composition was determined twice weekly. Three times during the trial (wk0, 6, 11) body fat depots via ultrasonography (Raschka et al., (2016)) and liver fat content via gravimetrical method (Starke et al., (2010)) were determined (n = 16). Statistical analyses were performed using SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC). For the repeated measures, the PROC. MIXED procedure was used. The model contained wk and group as fixed factors as well as their interactions. For individual variation factors, time was set as repeated statement. For non-parametric data as body and liver fat, the Mann–Whitney-U-test (Dell Statistica 64, 2016, version 13) was used.

Results: With the beginning of transition, a lower milk production, BW and BCS as well as an increase in serum NEFA and BHB concentrations as indicators of energy status were observed in PG compared to the initial values. Minor changes in body fat depots occurred. The liver fat content of the PG slightly increased from wk 1 to 6 in comparison to the CG and decreased afterwards. Despite a higher concentrate supplementation during fulltime grazing, a decrease in milk production from wk 4 on could not be prevented. Nevertheless, from wk 6 onwards, trends for elevated BW and BCS were observed. Furthermore, from wk 6 and 7 on a decreasing in serum BHB and NEFA concentrations during fulltime grazing could be documented.

Conclusion: During fulltime grazing, data show that due a higher amount of concentrate feed an energy deficit could be only counteracted to a certain degree as indicated by minor changes in fat depot masses and serum parameters, indicative for energy metabolism.

This study was supported by the MWK (Niedersächsisches Ministerium für Wissenschaft und Kultur).

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107.

Comparison of two *In vitro* systems for the estimation of total gas production and utilisable crude protein at the duodenum from native or ensiled field peas and field beans in ruminants

Vergleich zweier In vitro Systeme zur Schätzung der Gasproduktion und des nutzbaren Rohproteins am Duodenum aus nativen oder silierten Futtererbsen und Ackerbohnen bei Wiederkäuern

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In laboratory practice, attempts are made to automatize analytical processes. For *In vitro* evaluations of gas production (GP) and utilisable crude protein at the duodenum (uCP) from feeds, electronic pressure transducer techniques were applied. The ANKOM^{RF} Gas Production System is a gas pressure monitoring system (GPMS) that operates with automated measurements in vented bottles and wireless data transfer, promising significant savings in labour and time. GP and uCP obtained from the GPMS were compared to those obtained with the modified Hohenheim Gas Test (mHGT). We hypothesized that no or systematic differences exist between the systems.

Methods: Native and ensiled field peas (FP) and field beans (FB), maize starch and cellulose were used as substrates. In contrast to previous tests (1), the pH was not lowered as required during ensiling (pH 6.5 and 6.4 after 60 d ensiling of FP and FB, respectively), but we used the terms „ensiled" or „ensiling" to indicate the target preservation method. Within each of six consecutive trials, both systems were run at the same time, with the same inoculum and distribution of substrates, blanks (only inoculum) and standards. Ruminal fluid was taken from two cannulated wethers, filtered and mixed by 1:2 with a buffer/nutrient solution (2), in which NH_4HCO_3 was added at 2 g/L and $NaHCO_3$ was reduced by 2 g/L. 0.2 g of ground substrates or standards (~1 mm) and 30 mL of inoculum were applied to each fermentation unit. mHGT flasks and GPMS bottles, the latter each capped with a gas pressure measuring module, were incubated at 39°C under continuous agitation. GPMS settings were: 1 min recording interval; 1.5 psi threshold for releasing accumulated gases; 150 ms valve open time. After 24 h, GP was documented and corrected for blanks and standard factors (only mHGT). Gas pressures were converted to mL of produced gas. Samples were taken after 8 and 24 h for NH_3-N analysis and uCP estimation. Statistical analysis was performed using SAS 9.4 MIXED with fixed system, substrate and measuring time effects, interactions and a random trial effect at a significance level of $P<0.05$. A regression analysis examined linear relations between GP or uCP from both systems.

Results: Total GP was lower in the GPMS than in the mHGT ($P<0.001$) with a slope of the linear regression between the methods of 0.998. Differences were found between substrates ($P<0.05$). GP ranking was equal with both systems ($R^2=0.57$). Starch had the highest GP, followed by FP treatments and cellulose, whereas FB treatments had the lowest GP. Estimated uCP differed between the systems ($P<0.05$). Treatments of FB had higher uCP contents than those of FP ($P<0.05$). The ranking of uCP among substrates was equal with both systems ($R^2=0.76$ and 0.57 at 8 and 24 h, respectively). uCP did not differ between the measuring times within a substrate using the mHGT, but lower uCP estimates were obtained after 24 h in the GPMS ($P<0.05$). Ensiling mostly had no effect on GP and uCP.

Conclusion: The GPMS is suitable to describe relative differences in GP and uCP contents of different feeds and automatically offers detailed GP kinetics with less than a 1 min resolution. It may obviously reduce analytical efforts in labs, but, compared to the mHGT, absolute GP and uCP data would need correction. This GPMS should be validated using a larger number of different substrates.

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

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108.

Investigations on thiamine contents of organically produced cereals and grain legumes for poultry diets

Untersuchungen zu Thiamingehalten in ökologisch angebauten Getreiden und Körnerleguminosen für Geflügelrationen

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Thiamine deficiency leads to major depression of performance and health. Thus, it is common to supplement thiamine in diets for poultry. However, since genetically modified organisms (GMO) are used to produce B vitamin supplements, their use is restricted in organic farming. Thus, we aimed to find out if common diets can supply sufficient amounts of thiamine for poultry in organic farming.

Methods: During three years (2011 – 2013), a total of 855 samples of cereals and legumes 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 analysed for their contents of thiamine using a modified HPLC-method with FLD detection (1). We calculated minimum and maximum total content of thiamine in exemplary cereal based diets for starter chicken, broiler chicken, and laying hens in organic farming (2, Table 1) using own analytical data for wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), field peas (*Pisum sativum* L.), and field beans (*Vicia faba* L.) as well as table values (3) for other components.

Table 1: Amount of single feedstuffs [g/100g DM] in exemplary cereal based diets for chicken

	Wheat	Barley	Oats	Peas	Beans	Green Meal	Maize Gluten	Brewer‘s Yeast	Oil and Minerals
Starter	46			20	8		17	2	7
Broiler	35	10	10	7	15	5	10	3	5
Layer	35	5	10	15	6	3	12	2	12

Results: Thiamine contents varied widely especially in grain legumes (Figure).

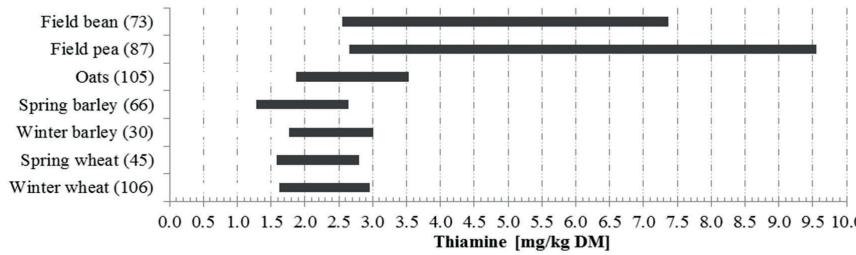

Figure: Ranges of thiamine contents in a selection of different grains

Even when only minimum contents of thiamine in cereal grains and grain legumes were assumed, the recommended amount of thiamine (in mg/kg DM of the whole diet) was met (Table 2).

Table 2: Recommended and actual amounts of thiamine in exemplary cereal based diets for chicken

Thiamine [mg/kg DM] in diets for	Starter Chicken	Broiler Chicken	Laying Hens
Recommended amount (GfE 1999)	1.9	2.8	1.7
Amount in exemplary diets	3.3 – 5.7	4.4 – 6.4	3.3 – 5.4

Conclusions: The results indicate that the thiamine supply of common cereal-based diets used for poultry feeding in organic farming is sufficient. A general supplementation of thiamine seems therefore not necessary. However, thiamine availability must be considered.

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109.

Comparative evaluation of dietary energy concentration and breed on feed intake and growth performance of fattening bulls

Vergleichende Untersuchungen zum Einfluss der Energiekonzentration der Ration und der Rasse auf Futteraufnahme und Leistung in der Bullenmast

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

Methods: 36 FV (age: 158d, body weight (BW): 222kg) and 37 BV (age: 151d, BW: 217kg) bulls were allocated by body weight and age at start of the trial to subgroups „energy norm" and energy high". Bulls were fed for ad libitum intake Total Mixed Rations (TMR) based on maize silage, maize corn silage, concentrates, and straw. Variation of energy concentration of TMR of groups energy norm and energy high (11.5 vs. 12.1 MJ ME/kg DM) was obtained by variation of portion of straw and rape oil in the TMR, respectively. Portion of concentrates differed only slightly among diets. Energy and nutrient concentration of the TMR was adapted to varying requirement over growth in 3 phases. Individual feed intake was automatically recorded daily while BW was recorded every four weeks. The bulls were slaughtered according to age in two subgroups having a slaughter age of 448 or 481d, respectively. For the statistical analyses a mixed model in SAS was used. The model included the main effects breed, energy concentration of TMR, slaughter age, and interaction of the main effects. Data of 35 animals of each breed were used for statistical analysis. Level of significance was set to $p<0.05$ and results are presented as lsmeans ± SE.

Results: DM intake tended ($p=0.078$) to be higher in FV (9.58 kg/d; ±0.17) compared to BV (9.17 kg/d) bulls, but there was no influence of dietary energy concentration. Daily ME intake was slightly ($p=0.074$) higher in FV (113 MJ/d; ±2) compared to BV (108 MJ/d) bulls, but there was no influence of dietary energy concentration on ME intake (113 vs. 109 MJ/d). FV bulls had higher body weight at end of the experiment (752 kg; ±10) and daily gain (1729 g; ±27) than BV bulls (715 kg and 1591 g). There was no difference in end weight and daily gain in animals of group ME high (739 kg and 1675 g) compared to group ME norm (728 and 1645 g). Intake of MJ ME/kg of body weight tended to be higher ($p= 0.061$) in BV (68.2 MJ/kg; ±0.9) than in FV (65.7 MJ/kg) bulls, whereas there were minor differences between groups ME high and ME norm (67.6 vs. 66.3 MJ ME/kg). Weight of kidney fat was higher in BV (19.3 kg; ±0.6) than in FV (16.5 kg) bulls. Higher age at slaughter led to higher amounts of kidney fat whereby effects appear to be more pronounced in FV bulls. Fat classification and back fat depth at slaughter, however, exhibited only minor difference between breeds. Carcass classification (E=1,…, P=5) was better in FV (2.41; ±0.08) than in BV (3.54) bulls. In both breeds, carcass classification was slightly improved at higher slaughter age with effects appearing more pronounced in BV bulls.

Conclusions: Results of the present study confirm higher growth potential and better carcass conformation in FV compared to BV bulls. However, also BV bulls exhibited excellent growth rate of approximately 1.600 g/d. A variation of dietary energy concentration of 0.6 MJ ME/kg DM had only minor effects on growth performance, what may be due to the low effects on DM intake. Albeit ME intake per kg of body weight gain appears to be higher in BV than in FV bulls, more data a needed to conclude on possible impact on recommendations for energy supply for different breeds. At the present stage the data provide no evidence that age at slaughter should be reduced in BV bulls compared to FV bulls.

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110.

In vitro investigations on the protein value of untreated and cell-disrupted microalgae for ruminants

In vitro Untersuchungen zum Proteinwert von unaufgeschlossenen und aufgeschlossenen Mikroalgen beim Wiederkäuer

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Microalgae are discussed as an alternative protein source for farm animals as they can have high crude protein (CP) concentrations. However, CP concentration varies between species and can even be variable within species. Additionally, robust cell walls or cell coverings may limit accessibility of intracellular protein. Therefore, the objective of this study was to investigate the protein value of different microalgae genera for ruminants including effects of cell disruption.

Methods: The method of the Extended Hohenheim Gas Test [1] was used to assay commercially available microalgae products of the four genera *Arthrospira* (*n=2*), *Chlorella* (*n=7*), *Nannochloropsis (n=4)* and *Phaeodactylum* (*n=2*). A subset of each sample was treated with a stirred ball mill for cell disruption resulting in a total of 30 samples for the *In vitro* incubations. Each sample was incubated with and without addition of a carbohydrate mixture over 8 and 48 h in six-fold replication. The effective utilisable crude protein (eff-uCP) and the effective ruminally undegradable crude protein (eff-RUP) were estimated for different passage rates (k) by plotting uCP and RUP values (y) against the logarithm of the incubation time (x) in a linear regression model using PROC MIXED of SAS. A two-factorial analysis of variance was carried out with the algae genera (AG), cell disruption (CD) and their interaction (AG×CD) as the fixed effects. Significance was declared at $p<0.05$. The ruminal degradability of crude protein (degCP) was calculated as the difference between CP concentration and eff-RUP at k=5%/h.

Results: The investigated microalgae had a high level of eff-uCP and eff-RUP and a low degCP but there were considerable differences between and in some cases even within genera. The fixed effects of AG and CD were significant for all traits but there were no significant interactions. Means for eff-uCP and eff-RUP were highest in *Arthrospira* or *Chlorella* and lowest in *Phaeodactylum*. The degCP was lowest in *Nannochloropsis*. Cell disruption decreased eff-uCP and eff-RUP and increased degCP. A low microbial protein synthesis was indicated by a high percentage of eff-RUP on eff-uCP in all microalgae (86-96 %) which is in good agreement with a low gas production observed previously [2].

		***Arthrospira**(n=2)*		***Chlorella**(n=7)*		***Nannochloropsis**(n=4)*		***Phaeodactylum**(n=2)*		***p*-values**		
		Mean	Range	**Mean**	Range	**Mean**	Range	**Mean**	Range	**AG**	**CD**	**AG×CD**
Eff-uCP(g/kg DM)	ut	**385**	377-393	**383**	339-437	**313**	273-366	**277**	274-280	<0.001	0.030	0.244
	cd	**392**	372-412	**335**	320-365	**294**	256-326	**243**	238-248			
Eff-RUP(g/kg DM)	ut	**349**	346-351	**360**	319-417	**297**	257-345	**259**	247-270	<0.001	0.006	0.077
	cd	**356**	337-375	**299**	280-334	**271**	233-309	**219**	219-220			
degCP(%)	ut	**49**	46-53	**36**	25-49	**31**	27-35	**42**	42-42	<0.001	0.007	0.310
	cd	**48**	42-54	**47**	40-55	**36**	33-40	**51**	49-53			

ut: untreated; **cd:** cell-disrupted

Conclusions: Microalgae are characterised by high content of RUP which lets them appear a promising protein source especially in high performing animals. Nevertheless, attention must be given to differences between and within genera. In addition, further studies are necessary to evaluate the intestinal digestibility of the RUP of microalgae and how it is affected by cell disruption.

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This work was supported by a grant from the Ministry of Science, Research and the Arts of Baden-Württemberg as part of the BBW ForWerts Graduate Program.

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111.

In vitro starch degradation and effective undegraded starch of different feedstuffs for ruminants

In vitro Stärke Abbau und effektive unabgebaute Stärke verschiedener Futtermittel bei Wiederkäuerern

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Information on ruminal degradation and the amount of ruminal undegraded starch is important for formulating diets for ruminants. The ruminal *in situ* technique is normally used to measure the undegraded starch of feeds. However, because of methodological problems associated with the outflow of small particles and starch granules from the nylon bags without being degraded, the method has been criticized in the literature and its use has been limited under practical conditions. In line with this, *In vitro* techniques are not biased by particles losses. Therefore, the aim of the present study was to evaluate the potential of an *In vitro* technique for the determination of degradation and undegraded starch content of different starch feeds for cattle.

Methods: Thirteen sources of starch (grains and roots) were ground to pass a 1.0 mm sieve and used. The *In vitro* degradation of the starch was determined using the Hohenheim gas test. Samples (200 ± 1 mg, on FM) were incubated in duplicate for 2, 4, 8, 12, 24, 32, and 48 h. After incubation, analysis of the remaining starch in the syringes was determined after enzymatic hydrolysis and determination of D-glucose content by spectrophotometry. An exponential monophasic model was fitted to the starch degradation values as $Y = A\,(1 - \exp{-ct})$, where Y is the cumulative starch degradation (%), A is the potential starch degradation (%), c is the constant rate of starch degradation (%/h), and t is the time (h). The effective undegraded starch (%) was calculated as 100 – the effective degradability of starch (%) assuming passage rates (k) of 2, 5 and 8%/h. Effective degradability of starch was calculated as $(A \times c)/(c \times k)$. Data were analysed using ANOVA and Tukey´s test.

Results: The starch content of samples varied from 9.5 to 80 % of DM. Starch of corn, millet, and rice were totally degraded, whereas starch of oats bran was the least degraded (88 %) among samples. For passage rate of 8%/h, the undegraded starch varied from 37.3 to 53.7%. Barley, pea, rye and wheat supplied the highest amount of undegraded starch (> 50%), whereas oats and oats bran supplied the lowest (average 38%) among samples.

	Starch	Starch degradation parameters		Effective undegraded starch (% of starch)		
Sample	(% of DM)	A (%)	c (%/h)	2%/h	5%/h	8%/h
Barley	55.1	95.6abc	7.54d	24.5a	42.6a	53.7a
Cassava	78.5	92.1cd	13.5bc	19.9bcde	32.9cdef	42.3cde
Corn	67.5	100a	9.83cd	17.1de	34.0bcdef	45.1bcde
Millet	73.8	100a	8.43d	19.2bcde	37.3abcd	48.7abcd
Oats	42.9	94.5abc	15.1b	16.6e	29.1f	38.4e
Oats bran	9.53	88.1d	19.9a	20.1bcde	29.8ef	37.3e
Pea	45.8	98.5ab	8.34d	21.0abcd	38.9abc	50.2abc
Potato	72.5	97.7abc	12.2bcd	16.1e	30.8def	41.1de
Rice	80.3	100a	8.69d	18.7cde	36.6abcde	48.0abcd
Rye	59.8	96.3abc	9.00cd	22.1abc	39.2abc	50.1abc
Triticale	59.6	94.5abc	9.48cd	22.3abc	38.6abc	49.2abcd
Wheat	59	98.2ab	7.65d	22.7abc	41.2ab	52.5ab
Wheat bran	14.9	92.7bcd	9.68cd	23.4ab	39.2abc	49.6abc

a-fMeans in the same column with different letters differ significantly ($P < 0.05$).

Conclusion: The *In vitro* method used in this study is a fast, low cost and reliable technique for the determination of starch degradation. Moreover, the technique showed its ability for ranking feeds in terms of supply of the amount of undegraded starch for ruminants. Further studies for the standardisation and validation of the technique are also recommended.

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112.

Methodological studies on the comparison of wet sieve analyses of pelleted compound feed

Methodische Untersuchungen zum Vergleich nasser Siebanalysen von pelletiertem Mischfutter

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The knowledge of the particle size distribution (PSD) and thus of the „feed structure“ in compound feeds is of great interest i. a. against the background of the prevention of gastric ulcers in pig production. Determination of the PSD in non-compacted feed is executed by dry sieve analysis in accordance to the industrial norms DIN 66165-1 and DIN 66165-2. However, for determination of the PSD in compacted feed (pellet, extrudate) a wet sieving analysis is necessary. For this purpose a variety of different methods exists. The aim of the present study was to compare the results and validities ascertained with various wet sieving methods on the base of one identical pelleted feed. Furthermore, a standardized wet sieving method should be deduced, with the aim of a good repeatability and low influence of the operator.

Methods: For the analysis a pelleted compound feed for pigs (Ø 3 mm) was used. The determination of the PSD was carried out with test sieves according to DIN EN 3310-1, (mesh sizes of 3.15, 2.00, 1.40, 1.00, 0.80, 0.50, 0.40 and 0.20 mm). The sieve shaker (HAVER EML 200 Premium Remote, Haver & Boecker, Oelde, Germany), was provided with a full cone nozzle in the sieving head, which served as water inflow. Following methods for wet sieve analysis were compared: **M1:** Engberg et al. (2002), **M2:** Miladinovic (2009), **M3:** Kamphues et al. (2007) and **M4:** in-house method (Rostock). For the in-house method 70 g of sample material were used, which was softened for 1 h in 1000 ml deionized water and stirred after 30 min. Sieving time lasted 10 min, however only for the first 3 min with a water flow of 3.4 L/min. The amplitude was set at 2.00 mm. Finally the sieves were dried and weighed. For statistical evaluation of data a one-factorial ANOVA was performed using SPSS 22.0 with either DUNCAN test or DUNNETT T3 test as post-hoc test. Differences between groups were considered as significant for $p \leq 0.05$.

Results: The results of the PSD (proportion in %) are presented in the following table (5 replicates per method [n=5]; mean [± SD]).

particle fraction	M1	M2	M3	M4
> 1.00 mm	10.1^{a}(± 0.90)	12.5^{b}(± 0.39)	21.9^{c}(± 0.67)	10.6^{a}(± 0.33)
0.20 - 1.00 mm	18.0^{a}(± 0.71)	18.9^{a}(± 0.56)	25.5^{b}(± 1.90)	17.7^{a}(± 0.32)
< 0.20 mm	71.9^{a}(± 0.76)	68.6^{b}(± 0.94)	52.5^{c}(± 2.58)	71.7^{a}(± 0.05)

^{a,b,c} indicate significant differences between the methods ($p<0.05$)

Using M1 and M4 the lowest proportion of particles > 1.00 mm and 0.20 – 1.00 mm were generated. The results of method M3 showed the highest proportion of the coarse and medium particle fraction but the lowest portion of particles < 0.20 mm. Within the methods the results had low deviations, whereas M3 showed the highest variations. No differences could be observed between M1 and M4 in the three particle fractions ($p>0.05$). The results of the method M3 differed ($p<0.05$) to those of all other used methods.

Conclusion: The results show that the large number of different wet sieving methods can lead to different assessments of the PSD in one compound feed. The repeatability of the results within one method is satisfactory, whereas M3 showed slightly greater deviations. Probably the manual execution and therefore the influences of the performer are the reasons for greater deviations of the results. The method M2 was very time-consuming and impractical and sieves clogged and overflowed easily. With the method M1, parts of the sample material got stuck on the lid and at the upper segment of the sieves partially, which had to be washed off after the sieving process. The results of the method M4 were similar to M1 an M2, but the execution was more practical and easier. Method M4 showed the slightest deviations without influence of manual handling. This suggests a good practicability with good repeatability and low influence of the operator.

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113.

Impact of increasing amount of *Sainfoin* in diets of entire male pigs on growth performance, carcass characteristics, meat quality traits and boar taint levels in the backfat

Einfluss einer ansteigenden Zulage an Esparsette in Mastrationen von Jungebern auf die Wachstumsleistung, Schlachtkörper-, Fleischqualität und den Ebergeruch

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Castration of male piglets is currently standard practice used by pig farmers to avoid boar taint, an unpleasant off-flavour of pork from entire males (**EM**). Boar taint has been attributed to the accumulation of androstenone and skatole in the fat tissue (1). Androstenone level is principally determined by genetic factors and stage of puberty, whereas skatole level in addition to a genetic component and hormonal status of the pig, is also controlled by nutritional factors (2). In recent years initiatives to abandon surgical castration has been undertaken by key stakeholders in Europe. This has stimulated intensive research to find possible alternatives to reduce boar taint. Dietary secondary compounds have shown promising results in this respect. Recent findings suggested that hydrolysable tannins (**HT**) can impair development of accessory sex glands and by that influence boar taint in EM (3). Unknown is whether condensed tannins (**CT**) have a similar effects as HT. The goal of the study was to establish the impact of increasing levels of CT originating from Sainfoin on growth performance, carcass characteristics, meat quality traits and boar taint levels in backfat of EM.

Methods: For the experiment, 48 Swiss Large White EM (BW 24.8 ± 5.1 kg) were assigned within litter to 1 of 4 grower (25-60 kg BW) and finisher (60-105 kg BW) diets supplemented with 0 (**T0**), 5 (**T5**), 10 (**T10**) and 15% (**T15**) sainfoin, respectively. The isocaloric and isonitrogenous diets were formulated according to the Swiss feeding recommendations for swine. Pigs were reared in one pen equipped with 4 automatic feeders, which allowed to monitor individual daily feed intake. All pigs were weighed weekly. They had *ad libitum* access to feed and water. At 170 d of age, pigs were slaughtered and organ weight and carcass and meat quality traits were evaluated. Boar taint compounds were determined in backfat by HPLC analysis. Data were analysed with the mixed procedure of SAS using the experimental groups as fixed effects.

Results: The inclusion of CT had no negative effect on growth performance in the grower period. Although feed intake tended ($P=0.07$) to be 10.2% greater in T10 than T5, growth rate in the finisher period and feed efficiency in the grower and finisher period were similar among groups. Slaughter weight and hot carcass weight were not affected by the CT supplementation although T0 pigs had a 2.7% greater ($P<0.001$) carcass yield than T15 pigs. Due to 6.4% heavier ($P<0.05$) ham weights, lean meat percentage was 4.4% greater ($P<0.05$) in T5 than T10 pigs, with intermediate values for T0 and T15 pigs. Expressed as percentage of hot carcass weight, relative liver weight was greater (2.05 vs. 1.81%; $P<0.05$) in T15 than T0 pigs whereas the relative salivary weight tended ($P=0.08$) to be lower (0.09 vs. 0.12%) in T10 than T5 pigs. Intermediate values were observed in T5 and T10 pigs for relative liver weight and for T0 and T15 pigs for relative salivary weight. In general the levels of boar taint were low and for androstenone not affected by treatments. Skatole and indole levels were numerically lower by 44 and 33%, respectively, in T15 and T10 compared with T5 and T0.

Conclusion: Increasing levels of CT from Sainfoin numerically reduced the concentrations of boar taint compounds in the backfat without compromising the performance neither in the grower nor in the finisher period.

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114.

Colostrum supply in suckling piglets in herds with different serological *Salmonella* prevalence in piglet rearing

Kolostrumversorgung von Saugferkeln in Beständen mit einer unterschiedlichen serologischen Salmonellenprävalenz in der Ferkelaufzucht

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The evaluation of the established Salmonella-monitoring system in pork production of Germany shows that there is no further improvement in the serological *Salmonella* prevalence for several years. Increasing litter sizes observed in recent years with low birth weights at the same time makes an adequate colostrum supply to suckling piglets more difficult (2). An optimal colostrum supply, delivering colostral immunity to piglets, can reduce the risk of a *Salmonella* infection in piglets (3). This study tested the hypothesis, that modern piglet producing farms with a high farrowing rate and an increased *Salmonella* prevalence in piglet rearing show a more unfavorable colostrum supply in suckling piglets.

Methods: An association of 250 northern German piglet producing farms has been organizing a voluntary biannual health-status-monitoring on piglets (25 kg BW) since years. The monitoring includes an ELISA for *Salmonella* antibodies. On basis of these data 12 *Salmonella*-conspicuous and 12 *Salmonella*-inconspicuous farms were selected. These were similar in terms of hygiene, farm size and performance. Each farm was visited once 24-48 hours after the main farrowing day. On each farm 4 litters were sampled and 2 light-weight, 2 medium-weight and 2 heavy-weight piglets per litter were weighed and a blood sample was taken. The blood samples were tested for the colostrum supply by means of the Ig-Immunocrit-method (4). Furthermore, *Salmonella* antibody level was tested by Herdcheck® *Salmonella* ELISA (IDEXX Laboratories, Hoofddorp, The Netherlands) measured in OD (Optical Density). Differences between the groups were tested by using the t-test (normal distributed) and the one way ANOVA-test (normal distributed; significance level: $p<0.05$).

Results: In this field study, there was a significant difference in Immunocrit values between the *Salmonella*-inconspicuous farms and *Salmonella*-conspicuous farms for the low weight piglets. There was no significant difference between the farms for the factor body weight and *Salmonella* OD of sampled piglets.

Table 1: Body weight (BW) and Immunocrit value of the piglets 24-48 h post natum (p.n.) divided into light-, medium- and heavy-weight piglets

		body weight [kg]		**Immunocrit**	
		Salmonella-inconspicuous farms	*Salmonella*-conspicuous farms	*Salmonella*-inconspicuous farms	*Salmonella*-conspicuous farms
BW category	n-animals / BW category	88	96	88	96
Light-weight		1.05 (±0.25)	1.05 (±0.29)	0.100[a] (±0.04)	0.087[b] (±0.04)
Medium-weight		1.38 (±0.25)	1.36 (±0.27)	0.107 (±0.03)	0.098 (±0.03)
Heavy-weight		1.69 (±0.27)	1.78 (±0.31)	0.114 (±0.03)	0.111 (±0.03)

[a,b] averages differ significantly within a column ($p < 0.05$)

Conclusion: This study provides preliminary evidence that when comparing *Salmonella*-conspicuous farms and *Salmonella*-inconspicuous farms, colostrum supply could be a critical factor to be considered. The fact that there is no difference in the body weight of the two groups suggests that there may be differences in colostrum management. Further studies have to examine what causes the different colostrum supply in the respective farms and if there is an impact on the *Salmonella* seroprevalence at the time of slaughter.

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This study was supported by EIP-Agri (Agriculture & Innovation), European Agricultural Fund for Rural Development (Project 276 03 454 035 0521).

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115.

Influences of higher dietary crude fibre levels on gains, behavioural disorders as well as on weight of organs (gizzard/liver) in fattening turkeys

Einfluss höherer Rohfaser-Gehalte im Alleinfutter auf tägliche Zunahmen, Verhaltensstörungen (Federpicken und Kannibalismus) sowie auf die relative Organmasse (Muskelmagen, Leber) bei Mastputen

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Beak trimming is a common practice in poultry to reduce damages/losses due to feather pecking and cannibalism [1]. In 2015 the BMEL and stakeholders of poultry industry signed a voluntary agreement to stop beak trimming in layers and turkeys in Germany [2]. For years diverse research was done to enable husbandry of non-debeaked turkeys. Increasing amounts of crude fibre (XF) is an approach that might contribute to a reduction of cannibalism [3]. This field study was done to evaluate the feasibility of rearing non-debeaked turkeys by higher amounts of dietary crude fibre.

Methods: 5 fattening periods on four commercial fattening turkey farms in northwest Germany (farm 1 participated twice) were examined. First 3 periods took place in winter („W"), last 2 periods in spring („S"). On each farm there were two compartments in which 300-400 day-old turkey hens (breed B.U.T. 6, British United Turkeys) were reared under the same climate and animal care conditions. The turkeys of the control groups were beak trimmed and fed the farm's standard complete feed, while the others (project) were not beak trimmed and (from feeding period 2 on) fed a diet with 1.5-2 % points more XF (by the addition of e. g. oat hulls, beet pulp, extracted sunflower seed and/or soy hulls) than the control. The composition of both control and project diets differed between farms depending on feed producers. In addition the project groups had access to wheat offered in separate troughs up to 7.5 % of their daily feed intake. At four times (wk 3, 6, 10, 14) body weight of 30 randomly selected turkeys per group was determined. On the basis of the last weighing, mean daily body weight gain (DBWG) from day 1 was calculated for each group (statistic: t-test). Furthermore the plumage and skin condition of the chosen animals were scored for detecting feather losses and integument injuries. At two times (wk 10, 15) 6 turkeys per group were dissected for further investigations on the GIT (data not shown). Also liver and gizzard of these hens were weighed and the organ mass relative to the body weight averaged over all dissected turkeys was calculated (statistic: Wilcoxon rank sum test).

Results: XF levels varied between 24-35 (control) and 41-56 g/kg diet (project). Differences between both groups in one trial ranged from 12-27 g/kg respectively. The mean body weight gain varied between the farms. In general body weight gains of the controls were numerically higher than those of project groups - except on farm 1 in „S". Losses due to cannibalism ranged between 1-28 turkeys (project group), no losses were recorded in controls. Between 0-71 project poults had to be separated due to injuries, in contrast to 0-15 control individuals. Severity and depth of injuries were higher in groups of non-debeaked turkeys. At week 10 only there was a significant effect of dietary treatment on gizzard and liver weight, but not in week 15.

Table 1 Mean DBWG (g/day), *p=0.05

		age, d	control	project
W	farm 1	98	92.9±7.52	91.3±7.47
	farm 2	96	92.3±6.51	86.9±7.64*
	farm 3	96	87.1±6.65	84.5±7.03
S	farm 1	93	94.6±6.28	96.2±5.80
	farm 4	93	94.7±6.46	92.1±9.24

Table 2 *Relative* organ mass in turkeys, *p=0.05

[weeks] age	[% of body mass]			
	gizzard		liver	
	control	project	control	project
10	1.73±0.28	2.03±0.46*	1.69±0.19	1.56±0.11*
15	1.20±0.24	1.20±0.21	1.33±0.18	1.32±0.23

Conclusion: A higher fiber level in diets for turkeys resulted in lower body weights in comparison to established commercial ones. The modified diet enriched with XF did not result in any positive effect regarding feather pecking and cannibalism. As an additional finding in wk 10, organ mass of gizzard was higher in project groups probably as a result of higher XF levels in the diet. Further investigations need to be done to evaluate the differences of liver mass between groups at the age of 10 weeks and between the two times of dissection. The storage of glycogen could play a role in this context.

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116.

Contents of precaecally digestible crude protein and amino acids and the amino acid profile of the total and insoluble crude protein fraction of compound feeds for horses

Gehalt an praecaecal verdaulichem Rohprotein und Aminosäuren sowie Aminosäurenprofil im gesamten und unlöslichen Rohprotein von Mischfuttermitteln für Pferde

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Assessing the protein value of horse feed *via* precaecally digestible (pcd) crude protein (CP) and pcd essential amino acids (EAA) allows feeding horses more close to their actual need (1). An important precondition for this approach is the similarity of the EAA profile of protein fractions irrespective of their solubility (1). The aim of the study was to investigate whether this is applicable to compound feeds for horses of different categories.

Methods: Subjects of the study were 39 commercially available compound feeds (for broodmares n = 6, foals n = 11, and other horses n = 22) taken in 2015 and 2016 in Germany by the Verein Futtermitteltest e.V. (VFT) as part of its regular control work. The feedstuffs were analyzed for proximate nutrients and neutral detergent soluble crude protein (NDSCP) (2). Amino acids (AA) were analyzed in the feeds and filter residues, derived from the NDSCP analytical protocol (2), with and without previous oxidation and ion exchange chromatography. Tryptophan was determined by HPLC. The contents of neutral detergent insoluble crude protein (NDICP), pcdCP and pcdEAA in the feed were determined according to (1). Statistical comparison of means was performed by use of the Kruskal-Wallis-Test (SPSS version 21) and $P < 0.05$ was considered significant.

Results: The compound feeds for mares and foals were higher in CP, pcdCP and pcdEAA ($P < 0.05$; see Tab.) than the feeds for other horses. This also applied to the estimated praecaecal digestibility of CP, which was 72.7 ± 2.13% and 74.3 ± 4.07% in the compound feeds for mares and foals and therefore higher ($P < 0.05$) than in the compound feeds for other horses 67.2 ± 3.59%. Irrespective of the feed type, the profile of important EAA (Lys : Met : Cys : Thr : Trp) in the CP and NDICP was similar: CP, 1 : 0.36 : 0,41 : 0.92 : 0.31, and NDICP, 1 : 0.35 : 0.43 : 0.97 : 0.29 (all compound feeds).

	CP [g/kg DM]	pcdCP	pcdLys	pcdMet	pcdCys	pcdThr	pcdTrp
Mares	176[a]	128[a]	0.60[a]	0,17[a]	0.21[a]	0.48[a]	0.16[a]
	± 26.7	± 20.2	± 0.157	± 0.027	± 0.035	± 0.090	± 0.028
Foals	178[a]	133[a]	0.59[a]	0.21[a]	0.20[a]	0.50[a]	0.16[a]
	± 19.6	± 18.4	± 0.185	± 0.092	± 0.029	± 0.122	± 0.027
Other horses	120[b]	81[b]	0.29[b]	0.11[b]	0.13[b]	0.29[b]	0.10[b]
	± 14.7	± 13	± 0.077	± 0.022	± 0.021	± 0.056	± 0.056

Conclusion: The higher pcdCP and pcdEAA contents in compound feeds for brood mares and foals *vs* other horses correspond to the individual requirements. The similar AA profile in differently soluble CP fractions supports the chosen approache to estimate pcdEAA contents in horse feeds.

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117.

Comparison of the particle size distribution between offered pelleted compound feed and gastric chyme after intake by fattening pigs using different wet sieving methods

Vergleich der Partikelgrößenverteilung zwischen angebotenen pelletierten Mischfutter und Magenchymus nach Aufnahme durch Mastschweine mittels verschiedener Nasssiebmethoden

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The prevalence of alterations of the pars nonglandularis of stomach in slaughter pigs can be up to 80% and leads to economic losses (1). It is known that the feed structure has a major influence on gastric health. Against this background, the knowledge of the particle size distribution (PSD) in compound feed (COF) is of particular interest. Determination of the PSD in non-compacted COF is executed by dry sieve analysis in accordance to the industrial norms DIN 66165-1 and DIN 66165-2. However, for compacted COF (pellet, extrudate) a wet sieving analysis is necessary and thus a variety of different methods exists. Therefore, the aim of the present study was to investigate which procedure is most effective to reproduce the PSD of gastric chyme compared to PSD of offered COF. Methods: For the analysis a commercial pelleted COF for pigs (Ø 3 mm) was used. Samples of gastric chyme were taken from 5 slaughter pigs (Ø BW: 35.7 ± 2.37 kg). After a 12-hour fasting time animals were offered the pelleted COF ad libitum 3 h before slaughter. The feed intake of animals took place up to 30 min until slaughter. For determination of the PSD in gastric chyme (GC) as reference, the fresh material was given over the stationary sieve stack and manually poured with water over each screen level until no particles of the next finer fraction were present. Test sieves were in accordance to DIN EN 3310-1, with mesh sizes of 3.15, 2.00, 1.40, 1.00, 0.80, 0.50, 0.40 and 0.20 mm. For wet sieving of the pelleted COF, following methods were used and compared with the PSD of gastric chyme: 1: ENGBERG et al. (2002), 2: MILADINOVIC (2009), 3: KAMPHUES et al. (2007) and 4: in-house method (ROSTOCK). The sieve shaker (HAVER EML 200 Premium Remote, Haver & Boecker, Oelde, Germany), was provided with a full cone nozzle in the sieving head, which served as water inflow. For the in-house method 70 g of sample material were used, which was softened for 1 h in 1000 ml deionized water and stirred after 30 min. Sieving time lasted 10 min, however only for the first 3 min with a water flow of 3.4 L/min. The amplitude was set at 2.00 mm. Finally the sieves were dried and weighed. The results refer to the proportions in dry matter. For statistical evaluation of data (n=5 replicates per sieving) a one-factorial ANOVA was performed using SPSS 22.0 with either DUNCAN test or DUNNETT T3 test as post-hoc test. Differences between groups were considered as significant for $p \leq 0.05$. Results: The results of the differences of the PSD of GC to the PSD of the offered COF ascertained with wet sieve methods (M1-M4) are presented in the following figure. Positive deviations imply that the proportion is lower, negative deviations imply that the proportion is higher than the reference. For the proportion of particles > 3.15 - ≥ 0.20 mm M1, M2 and M4 showed on average deviations from + 0.60 to 0.73 %-points to the reference, M3 differed by about – 1.70 %-points. Particularly high deviations were found in the fraction < 0.20 mm. On average M1, M2, M4 deviated to the reference from – 4.78 to – 5.83 %-points, whereas M3 deviated by + 13.3 %-points. Conclusion: None of the results of PSD in COF determined with M1 - M4 accurately reflected the reference PSD of the GC. However, deviations of all methods for the fractions from 3.15 to 0.20 mm were low. On the other hand it can been seen, that the fine fraction in COF is considered to be lower than it is in the GC if evaluating the fraction < 0.20 mm using M3. M1, M2 and M4 estimate these fractions higher than they actually are. All in all the results show that the large number of methods can lead to different assessments of the PSD in COF, especially with regard to the fine fraction and its possible ulcerogenic potential for pigs. Therefore, current benchmarks would have to be re-evaluated and adjusted if necessary.

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118.

Effect of rumen nitrogen balance on *In vitro* gas production and microbial protein synthesis from different nitrogen sources

Einfluss der ruminalen Stickstoffbilanz auf die In vitro Gasbildung und mikrobielle Proteinsynthese bei unterschiedlichen Stickstoffquellen

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Supply of dietary nitrogen (N) and energy such that they are available simultaneously and in proportions required by rumen microbes is expected to optimize both, diet digestion and efficiency of microbial protein synthesis. However, the effect on ruminal fermentation and microbial growth may differ depending on different N and carbohydrate sources. Hence, the objectives were to understand the effects of different levels of rumen nitrogen balance (RNB) on *In vitro* rumen fermentation and microbial protein synthesis and to determine whether these effects differ depending on the source of dietary N.

Methods: Three N sources (i.e., wheat gluten, soy protein, casein) differing in rate of degradation were incubated at three RNB levels (i.e., 0, -5, -9 g/kg dry matter) using grass hay as forage and corn starch as main source of carbohydrate. In total, two grams (as-fed basis) of a substrate mixture were incubated with 200 ml of McDougall's buffer (1) and 100 ml rumen fluid 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 corn starch and N 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, yields of liquid-associated (LAM) and solid-associated microbial mass (SAM) (3), and total microbial protein synthesis (i.e., sum of N in LAM and SAM) were determined after 24 h. All treatments were incubated in duplicate on three different days. The main effects of RNB, N source, and the interactions between RNB and N source were tested using PROC MIXED in SAS 9.4 at a significance level of $P<0.05$ (n = 6; 2 replicates x 3 incubations).

Results: Linear increases in GP with decreasing RNB were observed, likely due to higher proportions of degradable carbohydrates in the substrate mixture (Table 1). Similarly, total SCFA concentrations increased with decreasing RNB, with a tendency observed for wheat gluten diets ($P=0.07$). The NH_3–N concentrations decreased with declining RNB for all N sources; however, only wheat gluten and soy protein diets showed a decrease in LAM yield and total microbial protein synthesis.

Table 1 Fermentation parameters and microbial protein synthesis at different levels of rumen nitrogen balances (RNB, g/kg dry matter (DM)) tested in three nitrogen (N) sources during 24 h of *In vitro* incubation (least squares means).

	N source									SEM	P-value		
	Wheat gluten			Soy protein			Casein				RNB	N	RNB x N
Measured RNB	-0.6	-5.1	-9.0	-0.7	-5.1	-8.6	-0.5	-5.3	-8.6				
GP (ml/g DM)	188^{b}	201^{ab}	209^{a}	175^{b}	191^{ab}	195^{a}	166^{b}	179^{ab}	187^{a}	2.96	<0.01	<0.01	0.99
SCFA (µmol/ml)	27.6	28.9	30.2	22.9^{b}	26.4^{a}	26.8^{a}	23.8^{b}	23.7^{b}	27.2^{a}	0.56	<0.01	<0.01	0.50
NH_3–N (mg/l)	75.6^{a}	51.1^{b}	28.3^{c}	62.0^{a}	32.2^{b}	20.8^{b}	66.9^{a}	50.6^{ab}	31.8^{b}	4.00	0.03	<0.01	0.69
LAM (mg N/g DM)	8.0^{a}	7.6^{ab}	6.8^{b}	7.8^{a}	7.4^{a}	6.4^{b}	7.3	7.2	6.9	0.13	<0.01	0.32	0.58
SAM (mg N/g DM)	1.06	0.95	0.89	0.91	0.79	0.60	0.66	0.56	0.67	0.07	0.64	0.18	0.93
MP (mg N/g DM of incubated substrate)	9.1^{a}	8.6^{ab}	7.7^{b}	8.7^{a}	8.2^{ab}	7.0^{b}	8.0	7.8	7.6	0.17	0.01	0.14	0.55

GP, gas production; LAM, liquid-associated microbial mass; MP; microbial protein; NH_3-N, ammonia-N; SAM, solid-associated microbial mass; SCFA, short-chain fatty acid; SEM, standard error of mean. Within the same row means for each N source with different superscripts differ significantly ($P<0.05$).

Conclusions: The RNB below -5.1 g/kg DM do not hamper *In vitro* carbohydrate fermentation irrespective of the N source, but reduce microbial protein synthesis. The effects are less pronounced in rapidly degradable N sources (i.e., casein) compared to slowly degradable N source. However, it cannot be resolved whether differences in RNB effects between N sources for parameters *In vitro* might be less pronounced *In vivo* with more continuous supply of energy and N from dietary and endogenous origin. Further research is thus needed to identify reasons for the effects and to validate the results *In vivo*.

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119.

Comparison of packing density and fermentation quality between shredlage and conventional corn silage

Vergleich von Verdichtung und Gärqualität von Shredlage und konventioneller Maissilage

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Introduction: Corn shredlage is a type of silage produced from whole-plant corn that has been processed by forage harvesters that are equipped with cross-grooved processing rolls and set for a longer chop length than typically used. The potential benefits of this relatively new harvesting technique is improved physical effectiveness of the fibre, while the corn kernels are still processed extensively enough to ensure complete digestion (1). Even though the amount of shredlage fed to dairy cows in Europe and North America increased over the last years, the effect of the shredlage system on ensiling quality of corn silage is still matter of discussion. As longer chopped silages tend to compact not as good as consistently short chopped silage, particularly the packing density of shredlage might be affected adversely by its larger and more unevenly distributed particle size. Consequences of decreased packing density are incomplete exclusion of oxygen from the silo, which can lead to an undesirable shift in fermentation acid pattern, higher substrate pH, and subsequent spoilage. Consequently, this study aimed to compare packing density and fermentation quality between corn shredlage and conventional corn silage.

Methods: This study was designed as survey between 10 shredlage and 10 conventional corn silage pits. Participating farms were located in the Weser-Ems-Region in Lower Saxony. To asses packing density under similar conditions, all sampled silos were packed on solid ground (asphalt or concrete) without sidewalls. Only corn silos put up in fall 2016 were part of the study. Sampling was conducted in August 2017. The face of each silo was sampled with an electric drill fitted with a cylindrical core sampler (inner diameter: 9.2 cm, total volume: 1895.3 cm^3) at five different locations. Three sampling locations were positioned along the vertical centerline (50 cm from the top, midsection, and 50 cm from the bottom) and two on the vertical line separating the first and second quarter (50 cm from the top, 50 cm from the bottom) of the silo face. Packing density (wet) and temperature (20 and 40 cm into the silo face) were measured on-site. In the laboratory, samples were analyzed for fermentation acids, pH, dry matter (DM), organic matter, neutral and acid detergent fibre, crude fat, and enzyme-soluble organic matter. Fermentation acids and ethanol were analyzed by HPLC. To compare packing density, pH, temperature, and concentration of fermentation acids between shredlage and conventional corn silage, data were analyzed using the Mixed Model procedure of SAS with shredlage and silage as fixed and farm as random effects. LS means were compared using the Tukey–Kramer method. Treatment effects were declared significant at $P \leq 0.05$.

Table 1 Packing density and fermentation quality parameters of shredlage and conventional corn silage.

Item[a]	Conventional silage	Shredlage	SEM[b]	P-value
Packing density, kg DM/m^3	226.1	209.7	5.51	0.05
Substrate pH	3.89	3.88	0.053	0.88
Lactic acid, % DM	3.04	3.66	0.370	0.25
Acetic acid, % DM	2.08	1.90	0.243	0.61
Ethanol, % DM	0.89	0.92	0.075	0.81

[a]Mean of five core samples/silo
[b]Standard error of the means

Results: Mean packing density of shredlage silos (209.7 kg/m^3; Table 1) was 16.4 kg/m^3 lower compared to conventional corn silage (226.1 kg/m^3, all DM basis; P=0.05). Changes in silo face temperature, pH, and concentration of lactic and acetic acid were not detected (P>0.10). Similarly, concentration of ethanol did not differ between shredlage and silage (P>0.10). Concentration of butyric acid was below 0.3% DM in all samples, with no difference between treatments (P>0.10).

Conclusion: Even though mean packing density of shredlage was lower compared to conventional silage, a negative impact of shredlage on ensiling quality was not detected. However, the sample size was relatively small and more on-farm survey data is needed to fully assess ensiling quality of shredlage. As mean packing density of shredlage can be expected to be lower compared to conventional silage, producers have to be advised to monitor compaction closely when preparing shredlage.

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120.

Roughage based diets for pregnant sows – apparent digestibility and nutritive value of whole plant silages of wheat and maize

Grundfutter-basierte Rationen für tragende Sauen – scheinbare Verdaulichkeit und Futterwert von Ganzpflanzensilagen von Mais und Weizen

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In former times pregnant sows often were fed roughages, silages and beets, complemented with concentrates and minerals, but in modern piggeries the relevance of „combined feeding“ has lost its either role. Technologically advanced liquid feeding systems facilitate to feed whole plant silage (WPS) supplemented with minerals and further feed materials as liquid diets. Thus the question of this study was whether the formula based on XF content to calculate the apparent digestibility applies to whole plant silages today.

Methods: Four non-pregnant sows (bw: 179-277 kg, lactation-No: 2-5) were kept in individual pens which allowed free movement and direct animal contact. All sows were fed diets on maintenance level, calculated on the metabolic body weight (bw)[1)]. A ration of concentrates supplemented with minerals was fed as basis, in the following phases a share of concentrates and minerals was substituted by whole plant maize silage (WPMS) resp. whole plant wheat silage (WPWS) in two consecutive trials (concentrates: 888g DM, assumed 14.4 MJ ME; WPMS: 396g DM, assumed 10.7 MJ ME[2)], 967g OM, 81g XP, 27g XL, 164g XF, 696g NfE; WPWS: 501g DM, assumed 7.2 MJ ME[3)], 913g OM, 73g XP, 16g XL, 234g XF, 589g NfE; per kg DM). After fourteen days of adaptation to the new silage based ration followed seven days of collecting all faeces and refusals of the offered ration[4)]. The apparent digestibility (aD) of WPS was calculated using the difference method.

Table 1: Calculation of isocaloric diets on the metabolic body weight

sow No	body weight (kg)	Energy requirement [1)]	basis trial as fed (g)	WPMS trial as fed (g)		WPWS trial as fed (g)	
			concentrates	concentrates	WPMS	concentrates	WPWS
1	275	29.7	2311	1191	3712	1719	2138
2	184	22.0	1708	880	2743	1270	1580
3	208	24.1	1875	966	3012	1395	1734
4	200	23.4	1820	938	2925	1355	1684

Results: The aD of the two WPS (WPWS/ WPMS) was strongly correlated to the XF content. The calculated values of aD_{OM} confirmed the expected values, precalculated with the regression formula of AXELSSON & NEHRING[5)]. During the trial phase the isocaloric diets were devoured so the total daily feed intake (DM) was as calculated (WPMS-trial: 2.15 ± 0.29 kg; WPWS-trial: 2.23± 0.26 kg). The rations included 57% WPMS resp. 40% WPWS related to the fed dry matter.

Table 2: Apparent digestibility of whole plant silages in non-pregnant sows (n=4)

	WPMS	WPWS
aD OM expected, % [5)]	64.6	52.7
aD OM, %	64.5^{a}±1.26	52.7^{b}±2.93
aD XP, %	58.5±3.66	51.0±5.38
aD XL, %	81.0^{a}±2,21	64.8^{b}±6.82
aD XF, %	24.9±3.37	27.7±2.57
aD NFE, %	73.9^{a}±0,89	62.4^{b}±2.54
Energy[6)], MJ ME/kg DM	11.0	8.28

Conclusion: The linear regression equation[5)] described the influence of crude fibre on the digestibility. Calculating the apparent digestibility of the organic matter as a function of the crude fibre content applies to the used whole plant silages. Considering the nutritive value, the feed intake of WPWS had to be higher, for equal energy intake, as intended for animal´s welfare.

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

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121.

Forage and protein use efficiency in dairy cows grazing a mixed grass-legume pasture and supplemented with concentrates differing in protein and starch content

Proteinverwertung von weidenden Milchkühen bei Supplementierung von Kraftfuttern mit unterschiedlichen Protein- und Stärkegehalten

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Adjusting intakes of protein and in particular of rumen-degradable protein is most effective in reducing total and especially urinary N excretion (1). The aim was to analyze the effects of a supplementation of concentrate mixtures (CM) differing in their concentrations of rumen-degradable crude protein and readily fermentable carbohydrates on feed intake, diet digestibility, milk performance, and nitrogen (N) partitioning in lactating dairy cows grazing a mixed grass-legume sward.

Methods: Three dietary treatments were tested with two groups of dairy cows during three experimental periods (14 d adaptation and 5 d sampling) following a 2 x 3 Youden square design. Each cow daily received 4.12 kg CM (as-fed basis) distributed over two equal meals in addition to maize silage and the grazing of an alfalfa-clover-rye grass sward. The three CM contained (as-fed basis): 4 kg/d of dried distillers' grains with solubles (DDGS) and no maize kernel meal (MM) (CM1), 2.5 kg/d DDGS and 1.5 kg/d MM (CM2), and 1 kg/d DDGS and 3 kg/d MM (CM3). Additionally, 0.12 kg/d of a mineral-vitamin mixture was added to each CM. Fecal excretion was determined using the external marker titanium dioxide (2) and apparent total tract digestibility of ingested organic matter (OM) estimated from crude protein concentration in fecal spot samples (3) in order to calculate daily OM and N intakes of cows. Data were analyzed using the mixed-model procedure of SAS with dietary treatment, period, and their interaction as fixed effects, and animal as random factor. Least squares means and standard error of the means (SEM) were calculated, and the significance level set to $P<0.05$.

Results: Total OM intake (12.0 kg/d, SEM 0.62, $P\geq0.44$) and OM intake during grazing (5.5 kg/d, SEM 0.35, $P\geq0.63$) did not differ between CM. However, digestibility of ingested OM and daily milk yield were lower for CM3 (0.726; 13.1 kg/d) than for CM1 (0.733; 13.8 kg/d) and CM2 (0.735; 13.8 kg/d) (SEM 0.180, $P\leq 0.031$; SEM 0.26 kg/d, $P\leq0.073$). Total N intake and urinary N excretion decreased from CM1 to CM2 and CM3 (Table 1). Accordingly, N use efficiency (in g milk N per g of N intake) was higher for CM3 than for CM1 and CM2.

Table 1 Nitrogen (N) partitioning and conversion efficiency in lactating dairy cows grazing an alfalfa-clover-rye- grass pasture and supplemented with three different concentrate mixtures (CM)[1] in the Andes of Peru during three experimental periods in summer 2015/2016 (least squares means).

Variables	**CM1**(n=16)	**CM2**(n=18)	**CM3**(n=18)	**SEM**	**P-value**[2]
N intake (g/d)	412[a]	373[b]	301[c]	19.6	≤0.010
RNB (g/kg OM)	5.0[a]	3.9[b]	0.1[c]	0.36	≤0.003
N excretion (g/d)					
Feces	97	95	95	4.9	≥0.58
Urine	238[a]	201[b]	133[c]	8.3	≤0.002
Milk protein (kg/d)	0.48	0.49	0.47	0.087	≥0.12
Milk N (g/g N intake)	0.19[c]	0.21[b]	0.25[a]	0.006	≤0.007

a,b,c Means within the same row with different superscripts are significantly different at $P<0.05$.

OM: organic matter; RNB: rumen nitrogen balance; SEM: standard error of the mean. [1]The CM contained (on fresh matter basis) 4 kg and 0 kg (CM1), 2.5 kg and 1.5 kg (CM2), and 1 kg and 3 kg (CM3) of dried distillers' grains with solubles and maize kernel meal, respectively; additionally 120 g of a mineral-vitamin mixture was added. [2]P-value from contrast tests.

Conclusions: Feeding CM low in rumen-degradable crude protein and rich in readily fermentable carbohydrates may slightly reduce diet digestibility and milk yield in dairy cows grazing mixed grass-legume swards, but can greatly improve N use efficiency and lower the risk of N emissions into the environment.

The study was partly funded by the German Academic Exchange Service (Grant No. 5715688).

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122.

Growth performance and nutrient contents of several insect species fed with grass, silage and cobs

Wachstumsleistung und Nährstoffgehalte von verschiedenen Insektenarten bei Verfütterung von Gras, Grassilage und Grascobs

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Edible insects are discussed to play an important role as foodstuffs or feedstuffs in the future. Generally fed with diets based on grain they compete with common livestock as pigs and poultry for feedstuffs, which can also be used directly by humans. With the aim to avoid such a feedstuff competition an experimental study was conducted. Fly larvae, mealworms, and grasshoppers were exclusively fed with grass, grass silage, or grass cobs to investigate, whether they accept such feedstuffs and achieve growth performance. Differences between the species with regard to feed acceptance and growth performance were expected.

Methods: The study involved a total of 2,700 fly larvae (*Hermetia illucens*), 2,700 mealworms (larvae of *Tenebrio molitor*), and 300 grasshoppers (*Schistocerca gregaria*), which were divided into groups of 300 (grasshoppers: 50) animals each. Always three groups of each species received either fresh grass, grassilage or soaked grascobs. Counts of animals, body weight, and feed consumption were recorded weekly. After 28 days animals were killed by freezing. Dry matter (DM), fiber fractions (NDF, ADF) crude protein (CP), and amino acid concentrations in animal bodies were analyzed. Data analysis included one-way ANOVA (SAS).

Results: As shown in the table below, considerable growth performance was achieved in fly larvae only with cobs. Mealworms gained best, grasshoppers only with fresh grass. Grasshoppers fed with cobs died within the first experimental week. Silage was totally refused. According to growth performance, feed consumption was highest for cobs and fresh grass. DM content of animals averaged 30 %, being significantly lower in fly larvae fed with soaked cobs. There was no difference in NDF contents between species, averaging 20 % in DM. Crude protein content in dry matter was high (53-70 % in DM), no matter which grass product was offered. Amino acid concentrations were similar in all insect species used and showed no effect of applied feedstuff.

Table: Growth performance and nutrient contents of insects

Insect species	fly larvae			mealworms			grass-hoppers
Feedstuff	grass	silage	cobs	grass	silage	cobs	grass
BW, start, g/box	2.1	2.3	1.9	21	23	23	3
BW, end, g/box	0.4a	1.3b	15.6c	26y	18z	17z	10
DM consumption, g/box	0.3a	0.0a	6.6b	3.2z	1.2z	13.2y	20
DM, %	49b	44b	15a	24	28	24	21
NDF, g/100 g DM	-	17	22	17zy	20y	15z	21
CP, g/100 g DM	-	62	53	71y	70y	67z	70
Lysine, g/100 g CP	-	4.8	5.1	4.6	5.1	4.5	4.4

a, b, c and y, z mark significantly ($p<0.05$) different means within one species of insects

Conclusion: At least some species of insects can be fed based on grass and grass products, avoiding competition in feedstuffs and nutrients to common monogastric livestock. However, peculiarities of specific insects concerning housing or hygienic conditions must be considered. Further investigations about nutrient requirements of insect species are necessary.

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123.

Feeding the Edible Meditaranean Field Cricket *Gryllus bymaculatus* and the Desert Locust *Schistocerca gregaria* Forskål on base of storable feed substrates derived from maize, soybean, cowpea and carrots

Fütterung der essbaren Mittelmeerfeldgrille Gryllus bymaculatus und der Wüstenheuschrecke Schistocerca gregaria Forskål auf Basis lagerbarer Futtermittel aus Mais, Soja, Augenbohne und Karotten

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The Mediterranean Field Cricket *Gryllus bymaculatus* and the Desert Locust *Schistocerca gregaria* Forskål are edible insects contributing to food protein in many human communities. However, they arise from natural catch or from rearing on base of solely fresh feed materials, which makes the systems vulnerable in terms of food security and food safety. Storable and hence controllable substrates could overcome this disadvantage. This pilot study focuses on the possibility to replace fresh by storable feed substrates for rearing these insects.
Methodology: Crickets and locusts were held in cages containing each 250-350 cricket nymphs (12 cages) or 220-330 locust nymphs (21 cages) upon hatching. Then, the animals were fed cage-wise different rations consisting of five storable substrates: corn meal, soybean extracts, cowpea leave, corn stover, and vitamin enriched, dried carrots. Cricket cages received a combination of corn meal and cowpea leave („Starch" diet, n=6) or soybean extracts and corn stover („Protein/Fiber" diet, n=6). Locust cages were fed the „Starch" (n=6) or „Protein/Fiber" (n=6) alone or in combination with vitamin enriched carrots („Protein/Fiber/Carrot", n=3; „Starch/Carrot", n=3), and a combination of cowpea leaves, soybean extracts and vitamin enriched carrots („Protein/Carrot", n=3). Total feed consumption, harvested insects and total excrements were monitored cage-wise and were analyzed for nutrient contents. Apparent digestibility of feed dry matter (DM) was estimated on base excrement DM. Statistics included ANOVA within insect species using feeding groups as treatments and individual cages as replicates.
Results: Crickets gained biomass well when fed on „Starch" but not with „Protein/Fiber". Locusts fed on „Starch" and „Protein/Fiber" failed to perform. Adding carrots, locusts fed on „Starch/Carrot" still failed to grow while gained biomass with „Protein/Fiber/Carrot". Crickets transformed feed DM into body DM more efficient when fed on „Starch" than with „Protein/Fiber" (15% vs. 10%, $P<0.01$). Digestibility of feed DM was higher with „Starch" than with „Protein/Fiber" (55 vs. 42%, $P<0.01$) whereas conversion of digested feed DM into body DM showed the same efficiency. Feed nitrogen was transformed more efficient in crickets fed on „Starch" compared to „Protein/Fiber" (43 vs. 27%, $P<0.001$). For locusts, feed DM transformation was more efficient with „Protein/Fiber/Carrot" (16%) and „Protein/Carrot" (20%) than with „Starch" (11%, $P<0.01$) whereas DM digestibility did not differ. Transformation of digested feed DM into body DM was less efficient with „Starch" (16%) than with „Protein/Carrot" (39%, $P<0.05$) and „Protein/Fiber/Carrot" (28%). Obviously, locusts fed on „Starch" could digest the diet but could not utilize the nutrients efficiently for growth (e.g. due to protein deficiency). Corresponding nitrogen transformation of „Protein/Fiber/Carrot" was superior to starch (49% vs. 36%, $P<0.05$).
Conclusion: The insects accepted the offered substrates as feed. Therefore, these substrates may replace fresh feed materials and may thus improve efficiency and safety of insect production systems. Indeed, certain feedstuff combinations revealed nutritional limitations. They might serve as model diets to derive requirements e.g. for protein, amino acids, vitamins, etc.

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124.

Influence of different energy, protein and NDF contents in the diet on the growth of broiler chickens

Einfluss einer unterschiedlichen Konzentration an Energie, Protein und NDF im Futter auf das Wachstum von Mastbroilern

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Soluble and insoluble fibers might influence gut morphology and function of birds. Neutral Detergent Fiber (NDF) measures most of structural components in plant cells. It is recommended to use 35 g crude fiber per kg in broiler starter feed and with older broilers 40-45 g/kg (1). The following study was carried out to investigate the influence of increasing proportions of targeted NDF/crude fiber contents in diets (Table 1), either a low or high level of crude protein and energy (ME) on the growth performance of broilers.

Methods: 600 one-day old male chickens (ROSS) were randomly distributed into 6 treatment groups (Table 2) with 10 pens per group over a study period of 42 days. FeedIt is recommended to use 35 g crude fiber per kg in broiler starter feed and with older broilers 40-45 g/kg (1). 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. Data were analyzed via a two-way ANOVA (SAS).

Table 1 Main ingredients and analyzed nutrients of the diets (g/kg)

Feed stuffs/group	1	2	3	4	5	6
Corn/wheat	400/259	324/200	220/200	396/150	285/150	192/150
Wheat straw	10	52	97	7	48	90
Oat	20	50	50	-	-	-
Crude protein (CP)	191	194	187	217	213	217
Crude fiber (CF)	27	36	81	29	51	67
Neutral detergent fiber	97	116	185	90	131	159
ME, MJ/kg	11.80	11.80	11.80	12.80	12.80	12.80

Results: The results of this study indicate that an inclusion rate of approximately 50 g crude fiber in broilers" diet significantly increased the final body weight and decreased the feed to gain ratio (Table 2). The increasing CF content in the feed led in the bird to a proportionally increasing content of gizzard. The protein/energy–concentration in the feed had a significant influence on the carcass yield.

Table 2 Performance parameters of broilers; d1- d42 (means)

GroupCP/ME/CF	Feed intakeg/d	Final body weight, g	Feed to gain ratio, g/g	Carcass%	Gizzard%
1 – 195/11.8/27	116	3185	1.655	72.7	1.05
2 – 195/11.8/36	121	3520	1.547	72.9	1.15
3 – 195/11.8/81	122	3262	1.690	72.9	1.36
4 – 215/12.8/29	112	3510	1.449	73.7	1.01
5 – 215/ 12.8/51	112	3505	1.427	73.6	1.11
6 – 215/12.8/67	106	3250	1.477	74.5	1.31
Anova (p-values)					
Crude protein/ME	<0.001	0.007	<0.001	0.003	0.400
Crude fiber	0.200	<0.001	<0.001	0.500	<0.001
CP/ME x Crude fiber	<0.001	<0.001	0.030	0.600	0.900

Conclusion: The results showed that both the protein/energy concentration and the content of fiber in the diet are important for an optimal development of the broilers. In this trial a content of approximately 40-50 g crude fiber/kg feed seemed to be especially favorable for the development of the birds.

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125.

Effects of a partly defatted insect meal (*Hermetia illucens)* or micro algae (*Spirulina platensis*) in mixed diets on intestinal mucosal surface and mucin secretion of meat type chicken.

Einflüsse von teilentfettetem Insektenmehl (Hermetia illucens) oder Mikroalgen (Spirulina platensis) im Mischfutter auf Schleimhautoberfläche und Muzinsekretion im Dünndarm von Masthähnchen.

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Insects or algae are promising alternatives to replace soybean meal (SBM) in animal nutrition. As part of the multidisciplinary project „Sustainability transitions" the study aimed to investigate effects of 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 mixed chicken diets on mucosal surface and microstructure of the small intestine.

Methods: 180 one-day-old male growing chickens (Ross 308) were randomly allotted to 30 pens (6 birds per pen) for a growth study (34d) with three diets and feed/water supply on free choice level. The control starter/grower diet contained 39/32% SBM which was replaced in the experimental diets by HM and SM at 50% level with a basic AA fortification (AA added: Lys, Met) according to the control diet (1). After finishing the growth study, 8 birds per diet were slaughtered after 12 hours fastening and the small intestine was removed for stereological morphometry and histological analysis of the mucosa. A systematic uniform random (SUR) sampling scheme (2) was applied to 3 intestinal sections (I1: duodenum, I2: proximal jejunum I3: distal jejunum and ileum). From each section, length and weight was determined and 5 equal SUR sub-segments were collected after measuring their circumferences. All samples were formalin-fixed (4%), paraffin-embedded and cut into 4 µm vertical sections serially stained with hematoxylin eosin (HE) and Periodic acid-Schiff (PAS) reaction. The primary mucosal surface area (S_{pm}) was calculated by length x mean circumference of each intestinal section. For stereological estimation of the villus surface area (S_v), a villus amplification factor ($Ss_{(v,pm)}$) was determined in SUR generated visual fields from digitalized HE-stained slides with a stereological software tool (STEPanizer©). Mucin volume ($Vv_{(muc)}$) of the intestinal epithelium related to its basement membrane surface ($Ss_{(bm)}$) was stereologically estimated in SUR visual fields from digitalized PAS-stained slides. Statistical analysis utilized one-way ANOVA with Kruskal-Wallis multiple comparisons test (GraphPad Prism V5).

Results: Final body mass (BM) differed significantly ($p \leq 0.01$) between treatments (Control: 2439.7^{c} ±317g; HM: 1597.5^{b} ±105g; SM: 1195.2^{a} ±186g). Compared to control diet, the relative Spm was significantly increased in all gut sections with diet SM and in I1 with diet HM (Tab.). However, due to balancing effects by the villus amplification factor ($Ss_{(v,pm)}$) significant differences of relative S_v data were only observed between control and SM diet in I1. The mucin volume to surface ratio tended to be generally lower with diet HM.

	Control (n=8)			HM (n=8)			SM (n=8)		
	I1	I2	I3	I1	I2	I3	I1	I2	I3
relative S_{pm}(cm^2/g BM)	0.03^{a} (12%)	0.06^{a} (6%)	0.04^{a} (14%)	0.04^{b} (16%)	$0.07^{a,b}$ (13%)	$0.05^{a,b}$ (8%)	0.05^{b} (16%)	0.09^{b} (13%)	0.06^{b} (8%)
$Ss_{(v, pm)}$	19.1 (38%)	36.8 (30%)	23.1 (27%)	19.1 (19%)	29.1 (38%)	18.5 (46%)	17.7 (21%)	27.3 (27%)	20.2 (34%)
relative S_v(cm^2/g BM)	0.48^{a} (49%)	2.1 (31%)	0.95 (35%)	$0.69^{a,b}$ (31%)	2.06 (39%)	0.96 (46%)	0.80^{b} (20%)	2.5 (30%)	1.22 (35%)
$Vv_{(muc)}/Ss_{(bm)}$	3.75 (33%)	4.36 (32%)	7.44 (22%)	2.61 (37%)	3.85 (39%)	6.98 (31%)	3.33 (24%)	4.48 (43%)	8.32 (21%)

Mean values and coefficients of variation (CV) in %; means in the same row with different superscript letters are significantly different ($p \leq 0.05$).

Conclusion: Results indicate that algae meal based diets induce an increase in intestinal absorption surface, especially in the duodenum, possibly due to lower protein digestibility. The observed trend to lower mucosal mucin secretion following insect meal based diets might point to improved intestinal health. Further modifications of intestinal microstructure are under study in ongoing experiments.

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126.

Effects of soaking wheat and corn in lactic acid on the phytate-phosphorus and resistant starch content.

Der Effekt einer Milchsäurebehandlung von Weizen und Mais auf den Gehalt an Phytat-Phosphor und resistente Stärke

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Feed technological processes can increase the phosphorus (P) availability from phytate. Correspondingly, soaking of barley grains in 1% and 5% lactic acid released 0.5 to 1 g P from phytate (1). However, these processing methods may reduce the availability and concentration of other nutrients due to leakage into the soaking solution, thus lowering the nutritional value. In the present study, we evaluated the effect of soaking of wheat, corn and their combination in 1% and 2.5% lactic acid on the hydrolysis of phytate-P and starch concentration.

Methods: Corn (Zea mays), wheat (Triticum aestivum) and a 50:50 mixture of both (wheat-corn) were soaked in 0% (deionized water), 1% and 2.5% lactic acid solution for 0, 6, 12, 24 and 48 hours at room temperature (22°C) in three different runs with analyses performed in duplicates. After the respective soaking time, grains were separated from the remaining soaking solution and immediately freeze-dried before analysis. Total P, phytate-P, total starch, resistant starch (RS) and non-resistant starch (NRS) were measured were measured with enzymatic colorimetric assays. Results were analyzed with the Proc Mixed procedure of SAS taking into account the fixed effect of treatment and the random effect of the run.

Results: Total P content and the hydrolysis of phytate-P in the grains were affected by the type of cereal, lactic acid concentration and soaking time ($P < 0.05$). As such, the total P and phytate-P content linearly decreased with increasing soaking time and this decrease was more pronounced with 2.5% lactic acid than with 1% lactic acid or soaking in water ($P < 0.05$). Soaking in 2.5% lactic acid for 48 hours reduced ($P < 0.05$) the total P content by 16, 8 and 3% in corn, wheat-corn mixture and wheat, respectively. Furthermore, after soaking in 2.5% lactic acid for 48 hours, the phytate-P content decreased ($P < 0.05$) by 24, 25 and 30% in corn, wheat-corn mixture and wheat grains compared to 0 hour of incubation, thereby increasing the available P concentration in the cereal grains. In addition, soaking of the grains in lactic acid did not markedly affect the total starch content. However, soaking in 2.5% lactic acid increased the RS content in wheat by 51%, whereas it decreased the RS content in corn and wheat-corn mixture by 52 and 19%, respectively ($P < 0.05$).

Conclusion: Soaking of wheat, corn and a mixture of both in 2.5% lactic acid solution for 48% demonstrated to be an effective strategy to improve the phytate-P availability in the cereal grains. *In vivo* studies are necessary to evaluate whether the increase of RS in wheat grains after lactic acid soaking may reduce the energetic value of wheat.

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127.

Effects of feed intake level on efficiency of microbial protein synthesis and nitrogen balance in Boran steers

Einfluss des Futteraufnahmeniveaus von Boranrindern auf die Effizienz der mikrobiellen Proteinsynthese und Stickstoffbilanz

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Microbial protein synthesized in the rumen supplies > 50 % of ruminants" amino acid requirements (1), the share being particularly high in tropical environments where animals are often challenged by low feed intake and poor-quality diets. Hence, the aim was to explore how efficiency of rumen microbial protein synthesis (EMPS; in g microbial nitrogen (N)/kg digestible organic matter intake) and thus fecal and urinary N excretions respond to decreasing feed intake level and supplementation in tropical cattle fed poor-quality grass hay.

Methods: Twelve 18-months-old Boran steers (183 kg liveweight, standard deviation 15.2) were assigned to a 4 × 4 complete Latin Square design with 4 treatments tested in 4 periods. Each period comprised 3 weeks adaptation, 2 weeks sample collection (6 animals per sampling week), and 2 weeks of re-feeding in between periods. Steers were fed at 100 %, 80 %, 60 %, and 40 % of their metabolizable energy requirement for maintenance (MER) referred to as MER100, MER80, MER60, and MER40, respectively. Steers on MER80, MER60, and MER40 were only fed Rhodes grass hay. The MER100 steers were offered Rhodes grass hay at 80 % of MER as well as cottonseed meal and sugarcane molasses at each 10 % MER. Total feces and urine excretion were collected and analyzed for dry and organic matter (feces only), N, and purine derivatives (PD, urine only). Duodenal microbial N flow was estimated from urinary PD excretion (2). Data were analyzed using the mixed-model procedure of SAS. Additionally, linear effects of the three treatments only differing in intake level (i.e., MER80, MER60, MER40) were examined.

Results: Dry matter and organic matter intakes of steers increased with increasing feed intake ($P < 0.01$, Table 1). Despite differences in N intakes ($P < 0.01$), urinary N excretion ($P \geq 0.27$) and N balance ($P \geq 0.28$) did not differ between MER80, MER60, and MER40. Declining feed intake from MER100 to MER40 reduced duodenal microbial N flow ($P < 0.01$). The EMPS was greatest in MER100 steers compared to those receiving the other treatments ($P < 0.01$). The EMPS did not differ between MER80, MER60 and MER40 ($P \geq 0.42$) although a linear trend was detected when feed intake decreased from MER80 to MER40 ($P = 0.099$, Table 1). Similarly, total N and fecal N excretion declined linearly with decreasing feed intake ($P < 0.01$). The N intake, fecal N excretion, and N balance were greatest with MER100 amongst all treatments ($P < 0.01$).

Table 1 Nitrogen (N) balance and efficiency of rumen microbial protein synthesis (EMPS) in Boran steers at four feed intake levels (in % of metabolizable energy requirements for maintenance; MER) (least squares means, standard errors of means (SEM), n = 12).

Variable	Unit	Feed intake level (% of MER)				SEM	*P*-value		
		40	60	80	100		ANOVA[1]	Linear[2]	Quadratic[2]
Nutrient intake	kg/d								
DM		2.1^{d}	3.1^{c}	3.7^{b}	4.7^{a}	0.08	< 0.01	< 0.01	0.02
OM		1.9^{d}	2.9^{c}	3.3^{b}	4.2^{a}	0.08	< 0.01	< 0.01	0.02
DOM		1.1^{d}	1.7^{c}	2.0^{b}	2.5^{a}	0.06	< 0.01	< 0.01	0.02
N balance	g/d								
N intake		11.1^{d}	16.5^{c}	20.3^{b}	42.6^{a}	0.45	< 0.01	< 0.01	0.12
Urinary N		9.4^{ab}	8.6^{b}	10.0^{ab}	11.4^{a}	0.56	0.01	0.55	0.14
Fecal N		9.8^{d}	14.2^{c}	16.4^{b}	25.0^{a}	0.61	< 0.01	< 0.01	0.10
Total N excretion		19.3^{d}	22.9^{c}	26.4^{b}	36.4^{a}	1.41	< 0.01	< 0.01	0.88
N balance		-8.2^{b}	-6.4^{b}	-6.0^{b}	6.2^{a}	0.75	< 0.01	0.08	0.34
LW change	g/d	-739	-344	-84	204	1.45	< 0.01	< 0.01	0.23
Duodenal microbial N flow									
Total PD excretion	mmol/d	29.3^{d}	36.8^{c}	41.6^{b}	52.8^{a}	0.89	< 0.01	< 0.01	0.18
Microbial N	g N/d	7.8^{d}	13.3^{c}	16.6^{b}	26.8^{a}	0.78	< 0.01	< 0.01	0.23
EMPS	g N/kg DOM intake	7.0^{b}	7.8^{b}	8.2^{b}	10.9^{a}	0.51	< 0.01	0.10	0.69

[a b c d] Means within the same row with different superscripts are significantly different ($P < 0.05$).
DM: dry matter; DOM: digestible OM; LW: liveweight; OM: organic matter; PD: purine derivatives. [1]*P*-values from analysis of variance for all four diets; [2]*P*-values from contrast test for three diets differing in intake level (i.e., 40 %, 60 %, and 80 % of MER).

Conclusions: Decreasing feed intake level greatly reduces duodenal microbial N flow and thus protein supply to cattle offered poor-quality tropical forage below their MER, which may aggravate the negative effects of low dietary nutrient and energy supply in periods of feed shortage.Further research is required to quantify EMPS to be able to optimize protein supply and use efficiency in tropical ruminants.

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128.

Impact of a ration change from a Total Mixed Ration to Pasture combined with concentrate supplementation on immune cell parameters of Dairy Cows

Einfluss eines Rationswechsels von einer totalen Mischration zu einer weidebasierten Fütterung mit Kraftfuttersupplementierung auf immunologische Zellparameter von Milchkühen

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The transition from a total mixed ration (**TMR**) to pasture in spring requires metabolic adaptations for the cow, which might have consequences for health and immunoreactivity (Schären et al., 2016). Therefore, the aim of the present study was to examine whether transition from confinement to pasture influences immune parameters, especially indices that refer to effects on oxidative stress.

Methods: In 2016, a pasture trial was performed with 43 German Holstein cows. The animals were divided into a pasture- (**PG**, n=22) and confinement group (**CG**, n=21). The CG received a TMR (35% maize silage, 35% grass silage, 30% concentrate, dry matter (**DM**) basis) during the whole experiment, whereas the PG was transitioned to pasture (week (wk)0 and 1: TMR-only, wk2: 3 h/day(d) on pasture, wk3 and 4: 12 h/d on pasture, wk5 to 11: pasture-only plus 4.5 kg concentrate/cow/d (DM)). Changes in reactive oxygen species (**ROS**) production as indicator for homeostatic cellular function of blood neutrophils (**GR**) were determined in wk0, 6, 11 (dihydrorhodamine 123 assay, **DHR**) in unstimulated and tetradecanoylphorbol (**TPA**) stimulated GR (%). The mean fluorescence intensity (**MFI**) quantifies the mean conversion of DHR per cell (basal and stimulated with TPA). Furthermore, glutathione superoxide dismutase activity (**GPx**, **SOD** assay) and vitamin E (α-Tocopherol) concentration in blood serum (HPLC-Diodenarray detector) were recorded (wk0, 6, 11). In addition, blood cell differentiation (Celltac α MEK-6450, Nihon Kohden Corporation, Tokyo, Japan) was documented (wk0 to 11). Statistical analyses were performed using SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC). For repeated measures, the PROC.MIXED procedure was used. The model contained wk (T) and group (G) as fixed factors as well as their interactions (GxT). For individual variation factors, time was set as repeated statement.

Results: The concentrations of GR in blood increased from wk0 to 11 in PG. In addition, unstimulated GR of PG showed a stronger increase in ROS producing cells from wk0 to 11 compared to the CG ($PGxT<0.05$). The proportion of TPA-stimulated ROS producing cells amounted to 97.5 ± 2% in PG, and to 99.5 ± 0.3% in CG during the trial (LSmean ± SD). The basal MFI was not affected by the experimental treatment. The MFI of stimulated GR increased to 111% in CG while it increased to 147% compared to the initial values (wk0) in PG. Serum vitamin E concentration increased in PG until the trials end (PGxT<0.001). The GPx and SOD activity showed in PG no marked changes due experimental treatment (GPx: PGxT<0.01; SOD: PGxT=0.108).

Conclusion: Transition from TMR to pasture was associated with an increase in GR concentrations and modified proportions of ROS producing stimulated and unstimulated GR. The changes in unstimulated ROS production can be interpreted as a modification in basal redox-homeostasis within GR. Whether the improved vitamin E-status in PG is related to the observed effects in GR needs to be clarified further.

This study was supported by the MWK (Nieders. Ministerium für Wissenschaft und Kultur).

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129.

Ruminal crude protein degradation of hydrothermally processed full fat soybeans

Ruminaler Rohproteinabbau von hydrothermisch behandelten Vollfett-Sojabohnen

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European production of soybeans is increasing. Full fat soybeans (FFSB) are considered a potential alternative to soybean meal in rations for ruminants, but ruminally undegraded crude protein (RUP) content of FFSB is lower than in soybean meal. Hydrothermal treatment can be applied to increase RUP, but hydrothermal treatment requires energy input. The objective of this study was to explore how different treatment conditions change the RUP content of FFSB.

Methods: One batch of FFSB (40% crude protein (CP) and 22% crude fat in DM) was split into 8 treatments, which were hydrothermally treated as described in detail before (1) and as summarised in the table. Briefly, the conditioning time and duration prior to an expanding step (15 s, 125°C) were varied. Following expanding, additional autoclaving over different timespans was applied. *In situ* incubations of ground samples (2 mm) were conducted in three lactating Jersey cows over 2, 4, 6, 8, 16, 24, 48 and 72 h. Incubation residues and original samples were analysed for CP concentration. Degradation data were corrected for particle losses and an exponential function was fitted to the data: $y = a + b \times (1 - e^{-c \times (t-lag)})$; where a = soluble fraction; b = potentially degradable fraction; c = degradation rate; lag = lag time. The effective degradability (ED) was calculated using a ruminal passage rate of 8%/h (2). Estimated values were analysed using a mixed model (SAS 9.3) including animal as random and treatment as fixed effects.

Results: Maximal CP degradation (a+b) was ≥ 99% in all samples. Hence, any decrease in the a-fraction of treated samples was accompanied by a proportional increase in the b-fraction. The a-fraction and ED decreased with an increase in conditioning duration and temperature and were further decreased by additional autoclaving. Initial lag-time and c were reduced by conditioning and expanding without a clear influence of conditioning duration and temperature. Lag time and c were not further changed by additional autoclaving. The RUP content increased from sample SB0 to SB3a, but was not further increased by longer autoclaving.

	SB0	SB1	SB2	SB3	SB3a	SB3b	SB3c	SB3d	SEM	*P*
Cond. (min) 80 °C	-	*1*	-	-	-	-	-	-		
100 °C	-	-	*6*	*16*	*16*	*16*	*16*	*16*		
Autocl. (min)	-	-	-	-	15	30	45	60		
a (%)	53	30	13	10	6	6	5	4	-	-
b (%)	46^{f}	70^{e}	86^{d}	90^{c}	93^{b}	93^{ab}	94^{ab}	95^{a}	0.3	***
Lag (h)	2.3^{a}	0.9^{d}	1.1^{cd}	1.4^{bc}	1.7^{b}	1.6^{bc}	1.4^{bc}	1.4^{bd}	0.35	**
c (%/h)	20^{a}	13^{b}	13^{b}	11^{bc}	11^{c}	10^{c}	9^{c}	9^{c}	0.8	***
ED (%)	81^{a}	70^{b}	63^{c}	57^{d}	52^{e}	51^{ef}	50^{f}	50^{ef}	0.8	***
RUP (g/kg DM)	92^{f}	111^{e}	129^{d}	133^{cd}	144^{ab}	141^{bc}	153^{a}	146^{ab}	3.1	***

*** < 0.001; ** < 0.01

Conclusion: The RUP content of FFSB was increased by conditioning and expanding, which was more apparent with increasing conditioning duration. Under conditioning and expanding conditions used here, additional 15 min autoclaving further reduced ED, but the effect was probably not big enough to justify the additional effort. Effects on intestinal digestibility of RUP still need to be evaluated.

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130.

Yeasts in liquid swine diets – identification, growth at different temperatures and gas formation potential

Hefen im Flüssigfutter für Schweine – Identifikation, Wachstum bei verschiedenen Temperaturen und Gasbildungsvermögen

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Yeasts pose a risk factor regarding hygiene in liquid diets associated with off-flavor and loss of nutrients. Diarrhea, inflation and sudden death in pigs fed liquid diets highly contaminated by active yeasts are discussed to be correlated with high counts of yeasts in liquid feed. Yeast differentiation was done to investigate which species are often found in liquid diets. The hypothesis of this study was that distinct species only are able to grow and to build gas at temperatures similar to ones present in the pigs` alimentary tract.

Material and Methods: In total 42 liquid feed samples were analysed. Yeasts were isolated and morphologically characterized on Sabouraud-Glucose-Agar (SAB, Thermo Fisher Scientific). Of the 42 feed samples 33 were submitted for diagnostic purposes, Liquid diets without silage (LD–S); the remaining 9 samples obtained from own field trials, contained additionally whole-plant corn-silage (up to 66 % DM; LD+S). Biochemical differentiation of yeast isolates was done by ID 32 C (BioMérieux); MALDI-TOF analyses were performed on microflex MALDI Biotyper (Bruker) with direct smear method (MALDI-D) and with formic acid-ethanol extraction (MALDI-Ex). Gas production of 44 selected yeast isolates was investigated using 10 µl yeast suspension (McF 0,3) in 100 ml SAB-Broth in an ANKOM RF Gas Production System.

Results and Discussion: In total 95 morphologically different yeast colonies (size, structure, color, growth characteristics on riceagar) were isolated. In each feed sample 1 to 4 different yeast species were found. MALDI-EX provided the most results (79 %) but for 73 % of the isolates ID 32 C-test (easy to do in every laboratory) and for 73 % of the isolates the cheaper and quicker DS-method would have been sufficient for getting the same results. In spite of different morphology, yeast identification led sometimes to the same result. This might be due to the parallel presence of anamorphic and teleomorphic stages of the yeasts in one sample. Yeasts that were diagnosed twice in the same sample were not considered in the evaluation of the numbers of yeast species that was found in the respective feed sample. LD-S contained mostly *Candida* (*C.*) *krusei* (22 %), *C. holmii* (12.5 %), *C. humilis* (8 %), *Saccharomyces* (*S.*) *cerevisiae* (8 %) and *C. lambica* (6 %). LD+S contained mostly: *C. lambica* (29 %), *C. krusei* (24 %), *C. holmii* (12 %) and *C. pelliculosa* (12 %). Nearly no growth at 37 °C on SAB within 48 h was observed in 14 yeast isolates (14.7 % in total; 13 % of yeasts (LD-S) and 20% of yeasts (LD+S)). Larger colonies at 37 °C compared to 25 °C were mostly found with *C. krusei* (13/19 isolates) and *S. cerevisiae* (4/5 isolates). Growth performance related to incubation temperature of all 95 yeast isolates is shown in table 1.

Tab. 1: Growth performance of the yeasts at different temperatures in % of yeast isolates

Growth performance	higher at 25 °C	25 °C = 37 °C	higher at 37 °C
70 yeast isolates (LD - S)	54.3	21.4	24.3
25 yeast isolates (LD + S)	84.0	4.0	12.0
Total (n = 95 isolates)	62.1	16.8	21.1

Maize silage for LD + S was presumably stored outdoors during winter and therefore yeasts may have adapted to these temperatures. Most of the tested *C. krusei* (7/12), only one *C. holmii* (1/6) and none of the tested *C. lambica* (0/5) and *Trichosporon* spp. (0/3) isolates were able to build high amounts of gas within 24 h incubation at 38 °C.

Conclusion: Due to the facts that the majority of *C. krusei* isolates was able to grow at 37 °C but also at 25 °C, to form high amounts of gas and that it is known to grow in low pH-values (pH 3.6) and to build biofilms, yeasts are predestinated to grow in liquid diets, to stay in the pipeline and therefore may cause digestive disorders in fattening pigs. Clearly more than half of all yeast isolates grew better at 25 °C than at 37°C, 14 isolates didn`t grow at 37°C at all, presuming that the latter could have only minor adverse effects in animals regarding their role for clinical problems.

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131.

How does the dietary inclusion of *Hermetia illucens* larvae meal affect the porcine intestinal microbiota?

Wie wirkt sich eine Rationszulage mit Hermetia illucens Larvenmehl auf die Zusammensetzung der Mikrobiota im Schwein aus?

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Pig production needs high amounts of protein feedstuffs usually obtained from plant derived protein sources. Insect protein meals could serve as an alternative protein source, but its use in nutrition is still in discussion due to a lack of knowledge about the mode of action and possible risk factors. This study was designed to investigate the effect of different inclusion levels of *Hermetia illucens* larvae meal in piglet rearing diets. A special emphasis was set on potential changes in microbiota composition along the gastrointestinal tract (GIT) as the increasing level of chitin may serve as an additional substrate for bacteria.

Methods: Weaned barrows with an initial body weight (BW) of 7.0 ± 0.4 kg were housed in floor-pens (4 pigs/pen) and allocated to one of four diets (n=20/diet) varying in the level of *Hermetia illucens* larvae meal (Hermetia Baruth GmbH, Baruth/Mark): control feed (CON), diet with 2.5% (HERM-2.5), 5% (HERM-5.0) or with 10% Hermetia meal (HERM-10). Larvae were raised on rye and wheat bran, defatted mechanically and ground, thus containing 12.5% crude fat and 57.9% crude protein (DM-basis) in the final product. Hermetia meal was included to the expense of soya protein concentrate and nutrient content was kept constant for all diets. Piglets were fed *ad libitum* for five weeks and performance (feed intake, weight gain, feed efficiency, faecal consistency) were determined weekly. After five weeks pigs (n=10/diet) were slaughtered, dissected and samples collected: intestinal wall from stomach, duodenum, proximal and mid-jejunum, proximal and terminal ileum, caecum and colon, chyme from stomach, small intestine (divided equally in 3 thirds), caecum and colon as well as feces. DNA was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedical) from a total of 600 samples. Illumina sequencing of the 16S rDNA [1] was used to characterize the overall bacterial diversity. Sequencing data were phylogenetically using the Mothur pipeline. Multivariate statistical analyses was done using PRIMER-E [1].

Results: There was no difference in performance between the animals fed with four different diets during a 35d-rearing period. 16S rRNA gene sequencing of all samples resulted in 1420 operational taxonomic units (OTUs) with 1319 OTUs commonly present in all sample. Although mucosa and chyme samples shared a high number of OTUs, microbiota structure of the sample types (chyme/mucosa/feces) was significantly different (p=0.001). The most abundant OTUs among all samples belonged to *Lactobacillus*, *Pseudoscardovia*, *Campylobacter*, *Bifidobacterium* and others. Bacterial sequences of the mucosa samples showed a significantly (p=0.001) higher diversity (H 2.8-4.5) than chyme and feces (H 1.8-4). Chyme samples showed reduced sequence diversity in the small intestine compared to the large intestine. When compared to CON diet, dietary supplementation with Hermetia larvae meal showed no significant effect on the overall microbiota similarity structure along the GIT. Only the sequence diversity within several mucosa sites and within chyme of the proximal third of the small intestine was significantly affected by dietary treatment using HERM-2.5 and HERM-10 compared to the CON diet. Furthermore, pairwise comparison between treatments revealed only in chyme samples of the small intestine significant dietary effects on the microbiota similarity structure. *Lactobacillus* was more abundant when animals were fed with herm compared to the CON diet. The opposite trend was found for *Pseudoscardovia, Bifidobacterium* and *Olsenella* in this section.

Conclusion: The overall obtained community composition and diversity of all sections and fraction of the GIT were comparable to what is known from current literature data. No overall effect of the insect protein meal was found on the bacterial composition, only minor changes in parts of the small intestine were observed, both in chyme and mucosa samples. The robust community composition among all dietary treatment was in line with the stable performance data of the piglets. Thus, the use of insect protein meal as dietary supplementation seems to be a suitable alternative in pig nutrition.

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132.

Effect of feeding level and fecal microbiota transplant on serum parameters in chickens of diverging feed efficiency

Zum Einfluss des Fütterungsniveaus und eines fäkalen Mikrobiota-Transplants auf Serumparameter bei unterschiedlich futtereffizienten Masthühnern

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Chickens of good and poor feed efficiency (FE) show FE-related variation in their feed intake, their gut microbiota and serum lipid profiles. Accordingly, differences in serum cholesterol and uric acid levels may represent serum markers for FE in chickens (1). By modifying the gut microbiota, nutritional intervention strategies may improve chicken FE, which may be reflected in the serum profiles of chickens. In the present study, we investigated the effect of the feeding level and the administration of a fecal microbiota transplant (FMT) prepared from excreta of chickens of good FE on serum profiles and the efficacy of these nutritional strategies to improve chicken"s FE.

Methods: Two experiments were conducted with 110 (Exp. 1) and 112 (Exp. 2) one-day-old broiler chickens each. Chickens were individually housed in cages from day 9 to 36 of life. In Exp. 1, half of the chickens were fed restrictively from day 9 of life (85% of *ad libitum* feeding group), whereas the other half had *ad libitum* access to feed. In Exp. 2, half of the chickens were inoculated with a FMT (104 colony forming units) into the esophagus, whereas the other half obtained a control transplant (CT, phosphate-buffered saline) on day 1, 6 and 9 of life. In Exp. 2, chickens had *ad libitum* access to feed. Feed intake and body weight were recorded weekly for individual chickens between day 9 and 30 of life for the calculation of the residual feed intake (RFI), which was used as metric for feed efficiency, using PROC REG of SAS. Chickens with extreme RFI values were stratified into high (HRFI; n=14/experiment) and low RFI (LRFI; n=14/experiment). Blood samples were collected from the *Vena jugularis* on the last experimental day and serum parameters were determined by standard enzymatic colorimetric analysis. Statistics were performed by ANOVA using the MIXED procedure of SAS with treatments as main effects and batch as random effect.

Results: In Exp. 1, restrictively-fed chickens ate on average 300 g less and gained 250 g less between day 9 and 30 of life than *ad libitum*-fed chickens ($P < 0.05$). Lowering the feed intake improved the RFI value of HRFI chickens by 50% compared to the *ad libitum*-fed HRFI chickens ($P < 0.001$). With respect to the blood profiles, serum urea was 18% lower in restrictively-fed chickens compared to *ad libitum*-fed chickens ($P = 0.02$). In contrast, restrictively-fed chickens tended ($P = 0.06$) to have 7%-higher serum cholesterol compared to *ad libitum*-fed chickens, whereas LRFI chickens had 11%-lower cholesterol levels than HRFI chickens ($P = 0.04$). In Exp. 2, the FMT did not affect chicken"s RFI, but tended ($P < 0.10$) to enhance the feed intake and body weight gain in females. Moreover, the FMT lowered serum triglycerides by 29% and raised serum alkaline phosphatase by 52% compared to the CT ($P < 0.05$).

Conclusion: Restrictive feeding may be an effective strategy, whereas the administration of a FMT from highly feed-efficient chickens proved less effective to improve the FE in chickens. FMT-related changes in gut colonization may have modified feeding behavior in female chickens. Both nutritional interventions caused changes in serum profiles, indicating alterations in the systemic metabolism likely due to reduced nutrient availability and differences in gut microbe-host-interactions.

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133.

Response to a diet composed of food industry by-products of late laying dual-purpose hens in comparison to layer hybrids

Reaktion von Zweinutzungshennen gegenüber Legehybriden in der späten Legephase auf ein Futter bestehend aus Nebenprodukten der Lebensmittelindustrie

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Commercial laying hen diets are mainly composed of cereals and soybean meal. Following the concept of ‚feed no food' and because of environmental concerns in soybean production, alternative energy and protein sources are sought. A laying hen diet composed of food industry by-products but without soy is expected to be of lesser quality. Dual-purpose types, from which the males are fattened for meat and females used for egg production, might tolerate such a diet better than specialized layer hybrids, especially in the late laying phase. As spent hens they may also have a higher carcass value than the layer hybrids. Therefore, the aim of the study was to compare the laying performance and carcass quality of one novel and two traditional dual-purpose types and a layer hybrid in the late laying phase fed either a commercial layer diet or a diet composed of food industry by-products without soybeans.

Methods: Thirty-eight individually kept hens of Lohmann Brown Plus (LB, layer hybrid, n=10), Lohmann Dual (LD, novel dual-purpose type, n=10) and the traditional dual-purpose types (n=9) Belgian Malines (BM) and Schweizer Huhn (CH) were investigated. The experimental feeding period lasted for 12 weeks when the hens were 43-54 weeks into laying. They were slaughtered afterwards. Half of the hens received the control diet (C) based on maize, soybean meal and wheat (11.5 MJ/kg metabolizable energy (AME_N); 168 g/kg crude protein (CP)). The other half was fed the ‚by-product' diet (E) based on broken rice, sweet lupine, wheat bran, brewer's grains, fava beans and rapeseed cake (10.4 MJ/kg AME_N; 183 g/kg CP). They had *ad libitum* access to feed and water. Body weight (BW), feed intake and laying performance were determined weekly. At slaughter, carcass, breast and leg meat were weighed and sausage yield was calculated as the sum of the latter two. Data were analysed using the GLM procedure of SAS (version 9.4) with type, diet and their interaction as fixed effects. Multiple comparisons among Least Square Means were made with the Tukey-Kramer option considering $P < 0.05$ as significant.

Results: Feed intake clearly decreased with E for LB and numerically for LD (Table 1). For CH and BM feed intake did not differ between C and E. Feed conversion ratio (FCR) differed only between hen types even though a numerically higher FCR occurred for E. The laying performance of BM and CH was low and did not differ between diets, whereas it decreased by 61 % in LB and 38 % in LD when fed diet E. Final body weight (BW) and carcass weight differed ($P < 0.05$) between hen and diet types. Sausage yield per bird was highest for BM (940 and 870 g) followed by CH (691 and 627 g), LD (547 and 505 g) and LB (437 and 387 g).

Table 1: Diet and type effects on performance

Type	Lohmann Brown Plus		Lohmann Dual		Schweizer Huhn		Belgian Malines		
Diet	C	E	C	E	C	E	C	E	SEM
Feed intake (g/d)	118^{a}	76^{c}	98^{abc}	81^{bc}	107^{ab}	122^{a}	109^{a}	109^{a}	6.3
FCR (g feed/g egg)	2.5^{b}	3.1^{ab}	2.8^{ab}	3.2^{ab}	4.5^{ab}	4.6^{ab}	5.0^{ab}	5.1^{a}	0.64
Laying performance (%)	94^{a}	37^{bc}	68^{ab}	42^{bc}	28^{c}	35^{bc}	18^{c}	25^{c}	9.0
Final BW (kg)	1.9^{cd}	1.6^{d}	1.9^{cd}	1.8^{cd}	2.8^{ab}	2.5^{bc}	3.4^{a}	3.0^{ab}	0.11

$^{a-d}$Means within a row carrying no common superscript are significantly different ($p < 0.05$).

Conclusion: Dual-purpose types tolerate better a low quality diet than specialized layers. The low yielding traditional types did not respond to diet quality at all and the commercial dual-purpose type reduced performance only to the level found with the layer hybrids. Unexpectedly, the sausage yield, i.e. the meat that can be used for food production from the spent hens, was not affected by the diet.

The Coop Research Program of the ETH Zurich World Food System Center and the ETH Foundation and the Swiss Federal Office of Agriculture funded this project.

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134.

Effects of glyphosate residues in feedingstuffs; and different concentrate feed proportions on immunological, hematological and oxidative stress parameters of dairy cows in established lactation

Einfluss von Glyphosatrückständen in Futtermitteln und unterschiedlichen Kraftfutteranteilen auf Immunparameter, das Blutbild und oxidativen Stress bei Milchkühen in der etablierten Laktation

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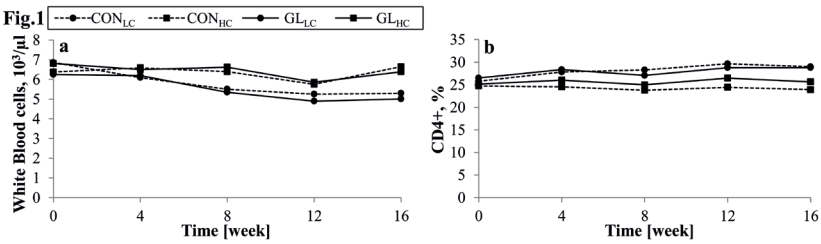
Glyphosate (GL) inhibits the aromatic amino acid biosynthesis in plants and is worldwide the most used non-selective herbicide. Less is known about *In vivo* immunological, hematological, and oxidative stress effects of GL in dairy cows. The aim of this study was to examine the effect of GL residues in feed, in combination with different concentrate feed proportions on hematology and redox-parameters of lactating dairy cows.

Methods: During a 17 weeks trial, 61 German Holstein cows (207 ± 49 days in milk; mean ± SD) were fed the same ration consisting of 30% maize silage, 30% grass silage and 40% concentrate (on a dry matter (DM) basis) in week 0 and were afterwards assigned to either a group receiving a GL contaminated total mixed ration (TMR) or a group receiving an uncontaminated TMR (CON). For this purpose wheat straw, wheat and peas with and without pre-harvest application with GL were used. 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). The diets were offered for *ad libitum* consumption. Blood samples were taken at weeks 0, 4, 8, 12 and 16. All blood samples were analyzed for white and red blood cell counts. For analysis of T-cell subpopulations and leucocyte functional assay (DHR-assay) a flow cytometer was used. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) were analyzed by enzyme assays. At week 16 the antioxidative capacity was measured by the ferric reducing ability of plasma (FRAP-assay). Data were analyzed using the MIXED procedure of SAS Enterprise Guide 6.1.; values represent the least square-means ± standard error.

Results: The average GL intake was 0.8 in CON groups, 73.8 in group GL_{LC} and 84.5 mg/d in group GL_{HC}. GL contamination did not affect performance (1) nor any of the tested parameters; whereas concentrate feed proportion (CFP) and time (t) showed interactions with higher values in HC groups for leucocytes ($p = 0.001$) (Fig.1, a), granulocytes ($p = 0.004$), red blood cells ($p = 0.006$), hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and CD4+T-cells ($p < 0.001$) (Fig.1. b). The same interaction was found in the DHR-assay, representing the maximal capacity for reactive oxygen species (ROS) production in granulocytes ($p = 0.007$), whereas the amount of stimulated ROS producing cells ($p = 0.031$) and GPx ($p = 0.020$) presented higher values in LC groups.



Conclusion: In the present study GL, and GL in combination with different CFP, showed no influence on immunological, hematological, and redox - parameters of lactating dairy cows, whereas the different CFP and time influenced most parameters in an interactive manner.

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135.

Impact of acute supplementary stress at slaughter on physiology and meat quality of fattening bulls

Einfluss von zusätzlichem akutem Stress kurz vor der Schlachtung auf Physiologie und Fleischqualität von Mastbullen

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During slaughter, cattle may be exposed to many potentially stressful events. The impact of acute pre-slaughter stress just before stunning on physiology and meat quality has not yet been exhaustively studied in beef cattle and its impact on meat quality might be underestimated (1). The study aimed at investigating the effect of physical and emotional stress at the slaughterhouse on physiology and meat quality of fattening bulls by means of two different slaughter protocols (SP).

Methods: 81 fattening bulls (Limousin × dairy breed) were reared under similar conditions from autumn 2016 to autumn 2017 at Agroscope Posieux, Switzerland. Before slaughter in a conventional slaughterhouse, animals were allocated to two SPs. SP A aimed at minimizing the stress by a two hour resting period in a quiet waiting pen after the arrival at the slaughterhouse until slaughter. Upon arrival, cattle of SP B were subjected to physical and emotional stress in the slaughter corridor during 30 minutes and subsequently slaughtered. Heart rate (HR) was measured by Polar® chest belts. Saliva was sampled after stunning, urine was directly collected from the bladder about 20 minutes after bleeding. Cortisol was analyzed by ELISA, catecholamines by HPLC/EC. Meat quality was evaluated on the Longissimus thoracis (LT) muscle. Statistical analyses were done using analysis of variance and Pearson correlations. In order to visualize multiple correlations, principal component analyses (PCA) were computed based on correlation matrices.

Results: SP B increased heart rate (+30%, $P<0.001$) during 30 minutes before slaughter, salivary cortisol concentration after stunning (+140%, $P<0.05$) and urinary concentrations of adrenaline (+33%, $P>0.1$), noradrenaline (+42%, $P<0.05$) and cortisol (+114%, $P<0.01$). The early post mortem pH decline was fastened for SP B for the first 6h ($P<0.05$), compared to SP A. Cattle of SP B had higher muscle lactate content (+1%, $P>0.1$) and temperature 1h post mortem (+2%, $P<0.05$). A PCA indicated that the physiological parameters as HR during 30 minutes before slaughter, salivary cortisol and urinary stress hormones were strongly correlated to each other. Further, all these variables were negatively correlated to the pH 1-6h post mortem. The PCA explained 58% of the total variability. SP B resulted in significantly lower meat juiciness. No effects of SP were found on ultimate pH or temperature (at 48h post mortem) or on Warner Bratzler shear force. Within the SP A, positive correlations were found between the HR at the moment of being in the stunning box and the water loss at cooking on day 14 of maturation ($r=0.4$, $P<0.05$) as well as the Warner Bratzler shear force at day 14 of maturation ($r=0.39$, $P<0.05$).

Conclusions: A supplementary physical and emotional stress just before stunning markedly affected physiological stress indicators as HR and stress related hormones in saliva and urine as well as early pH and juiciness.

Acknowledgements: The authors are grateful for the financial support of the foundation Sur-La-Croix, Switzerland.

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136.

Study on course of plasma levels of gastrointestinal hormones (glucose-dependent insulinotropic peptide [GIP], glucagon-like peptide 1 and 2 [GLP-1, GLP-2]) that are supposed to be involved in small intestinal elongation in juvenile pigs after experimentally induction of exocrine pancreatic insufficiency

Studie zur Plasmakonzentration gastrointestinalen Hormone (glukoseabhängiges insulinotropes Peptid [GIP], Glucagon-like Peptide 1 und 2 [GLP-1, GLP-2]) mit möglicher Bedeutung für die Elongation des Dünndarmes bei jungen Schweinen mit chirurgisch induzierter exokriner Pankreasinsuffizienz

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Introduction: The pancreatic duct ligated (PL) pig is an established model to study the effects of exocrine pancreatic insufficiency (EPI) on digestive processes [1]. In juvenile pigs induction of EPI resulted in a significant elongation of the small intestine [2;3]. Former studies indicate that GLP-2 (glucagon-like-peptide 2) may play an important role in this process [2] as it is highly relevant for small intestinal growth in neonatal pigs [4]. Earlier studies indicates that in PL-pigs either undigested nutrients within the gut lumen or increased production of short chain fatty acids caused higher GLP-2 levels. Because up to now information about the time needed for these changes had been missing, as in a former study GIP, GLP-1 and GLP-2 levels were only measured once at the end of the trial (during dissection), this study aimed to determine bi-weekly the plasma levels of these gastrointestinal hormones.

Materials and Methods: The study was performed in 8 female cross-bred pigs. 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 (pair feeding; last two weeks ad libitum). Blood samples were taken every second week. Analysis of GIP, GLP-1 and GLP-2 in plasma samples was performed in a lab of J.J. Holst at the University of Copenhagen. During dissection the length of the small intestine was measured.

Results: Body weight at slaughter was significantly higher in controls compared to PL-pigs (146 kg vs. 111 kg) while small intestinal length was significantly higher in PL-pigs (25.8 m vs. 20.7 m). Plasma concentrations of GLP-1 and GLP-2 were significantly higher in PL-pigs starting in week 19 of life (see table 1) while GIP concentrations differed only at day of dissection (with levels being higher in controls).

Table 1: Course of plasma concentrations (pM) of GIP, GLP-1 and GLP-2 during course of time in controls as well as PL-pigs. Significant differences ($p<0.05$; t-test) between groups at same time point are marked with different symbols

Discussion: Marked changes in gastrointestinal endocrinological status occured within 3 weeks after experimentally induction of EPI. As EPI results in a distinct maldigestion and malabsorption the amount of nutrients within the intestinal lumen is increased markedly which is a trigger for both gastrointestinal hormones. The fact that highest levels of GLP-1 and GLP-2 were observed in week 17 in both groups indicates that surgery seems to affect gastrointestinal hormones (which should be taken into account whenever sampling is performed shortly after surgery).

Conclusion: This study clearly shows that EPI markedly affects GLP-1 and GLP-2 very fast (at least within 3 weeks) after experimentally induction of EPI. Therefore, this study underlines the role of GLP-2 acting as a trigger of small intestinal elongation in case of maldigestion due to EPI.

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137.

Comparative histology of esophageal submucosal glands in pigs exposed to different diets and housing systems with regard to the incidence of gastric ulcers

Histologischer Vergleich der submukösen Ösophagusdrüsen von Schweinen bei unterschiedlicher Fütterung und Haltung im Hinblick auf das Vorkommen von Magenläsionen

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The submucosal glands in the esophagus produce a mucus consisting of water, electrolytes including bicarbonate ions (HCO3-), mucins [1], epidermal growth factor and prostaglandins [2]. This mucus protects the esophageal mucosa against mechanical irritation by feed [3] and possible acidic gastroduodenal reflux [4]. It was shown in mandibular glands of growing pigs that different diets can induce morphological changes [5]. Thus, the aim of the present study was to investigate if the secretion, the composition and/or the number of the esophageal glands change in response to different diets and housing and whether possible changes can be related to the occurrence of gastric lesions.

Methods: For this purpose, glandular tissue of the esophagus of 40 pigs with and without gastric lesions was compared histologically. Tissue samples (proximal, middle and distal part of each esophagus) were collected from pigs of four different groups: Pigs (n=22) fed a coarse meal diet, housed on straw litter; dissection at the age of 37, 48, 63, 82/85 or 120 days. Pigs (n=6) fed a pelleted diet, housed on straw litter; dissection at the age of 49 or 63 days. Pigs (n=6) fed a pelleted diet, housed without litter; dissection at the age of 49 or 63 days. Pigs (n=6) fed a ground pellets, housed without litter; dissection at the age of 49 or 63 days. The relative glandular area in % of total area of Tunica mucosa and Tela submucosa, was determined in hematoxylin-eosin (HE) stained sections at 25-fold magnification in five adjacent fields of vision. Furthermore, the number of acini was counted. In addition, mucus composition in glandular cells was investigated by using different histochemical staining methods, namely Periodic acid-Schiff reaction (PAS) and Alcian blue (AB) with pH 1 and pH 2. The relative amount of stained acini in % of the total number of acini was determined per section at a 100-fold magnification in one field of vision. Statistical analyses were done using the SAS Enterprise Guide 7.1.

Results: Morphological examination revealed a clear difference in the location of porcine esophageal glands confirming data from literature [6]. Large densely packed glands can be found in the Tela submucosa of the proximal part of the esophagus. In the middle part there are only sporadic glands while distally no glandular tissue was noticed. In addition, there was no significant difference in the relative glandular area between investigated groups or between animals with or without gastric ulcers. Concerning mucus composition, histochemistry revealed heterogeneous and individual staining results per group. Neutral mucins were stained pink using PAS, whereas sulfated mucins and acidic mucins stained blue by AB pH 1 (sulfated) and pH 2.5 (acid).

Group	1	2	3	4
Relative glandular area (%)	39 (4,4)	41,2 (4,5)	44,8 (7)	40,6 (6)
Neutral acini (%)	100	100	100	99,1 (2)
Sulfated acini (%)	43,4 (47)	51 (49)	32 (36)	57,2 (42)
Acid acini (%)	99,8 (1)	100	100	100

This table shows the average values (standard deviation) of the relative glandular area, neutal-, sulfated- and acid acini of the different groups in the proximal section.

Conclusion: The present study shows that the distribution pattern between neutral, acidic and sulfated mucins in esophageal glands is certainly different but seems to be independent from diet, housing or gastric health. In contrast to mandibular glands, present data so far revealed no obvious changes in esophageal gland histology, localization, secretions and number. This might e.g. be due to the trial period (maximum of 28 days) that could have been too short to result in detectable morphological changes in glands. Thus, further immunohistochemical experiments will be performed to detect possible differences for example in bicarbonate secretion as alternative responses to different conditions in feeding and housing.

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138.

Conjugation frequencies of ESBL-producing Escherichia coli donors and various Enterobacteriaceae recipients from poultry

Konjugationsrate ESBL-bildender Escherichia coli Donoren und unterschiedlicher Enterobacteriaceae Rezipienten vom Geflügel

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Introduction: Within livestock, poultry show the highest prevalence of extended-spectrum ß-lactamase(ESBL)-producing bacteria. Despite that antibiotic resistance does not have a direct impact on the health of the animals, a potential threat to public health arises when the ESBL-carrying plasmids are transferred to pathogens. Recent studies have shown the ability of feed additives, such as probiotics, to reduce the ESBL-load. Nevertheless, deeper understanding of their impact on conjugation is needed to evaluate the nutritional effect on the spreading of antibiotic resistance.

Methods: The five different ESBL-producing Escherichia coli (E. coli) strains E. coli ESBL 10682 (CTX-M-1), E. coli ESBL 10689 (TEM-52), E. coli ESBL 10708 (SHV-12), E. coli ESBL 10716 (CTX-M-15) and E. coli ESBL 10717 (CMY-2, TEM-1) were co-cultivated with six Enterobacteriaceae strains, commonly detected in poultry. Conjugation frequencies were obtained after 0, 2, 4, 6, 8 and 22 hours where transconjugants were identified by spreading the bacteria solution on double antibiotic MacConkey agar comprising cefotaxime chloramphenicol (E. coli IMT 20751/402, E. coli IMT11716), colistin (Serratia marcescens subsp. marcescens DSM 5570), nitrofurantoin (Enterobacter cloacae DSM 30060, Proteus mirabilis DSM 4479), or sulfamtehoxazole/trimethoprim (Salmonella Typhimurium DSM 30122). Conjugation frequency was calculated by dividing the number of transconjugants by the donor count. The trial was conducted in a double approach.

Results: Conjugation frequencies differed depending on donor, recipient and time of incubation. While conjugation was observed within 10 minutes for Enterobacter cloacae DSM 30060 with E. coli ESBL 10708 and E. coli ESBL 10717, for the remaining strains it was observed after 4 hours or later. No conjugation was observed for E. coli ESBL 10716 with Serratia marcescens subsp. marcescens DSM 5570, Enterobacter cloacae DSM 30060 and Proteus mirabilis DSM 4479. Similarly, the recipient strain Proteus mirabilis DSM4479 showed no conjugation with all given donors. Conjugation was low for E. coli ESBL 10717 and the E. coli recipients while high conjugation frequencies were observed with Enterobacter cloacae DSM 30060. E. coli ESBL 10708 showed no conjugation when co-cultivated with Serratia marcescens subsp. marcescens DSM 5570. The donor strain E. coli ESBL 10689 showed conjugation with all tested recipients and the highest conjugation frequencies of up to 10^5 transconjugants/donor were observed for this donor when co-cultivated with Salmonella Typhimurium DSM 30122. No conjugation was observed between E. coli ESBL 10689 and E. coli IMT 20751/402. Overall, relatively high conjugation rates of mainly 10^{-7} – 10^{-6} transconjugants/donor were observed. Conjugation frequencies differed not only between different bacteria genera and species but are also strain dependent.

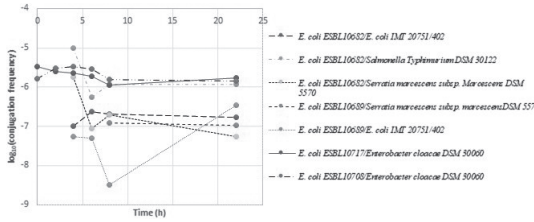

Figure 1: Logaryithmic visualization of conjugation frequencies (mean values)

Discussion: While conjugation in some cases only was observed after 22 hours incubation, it occurred within 10 minutes or not at all with other donor/recipient combinations. A possible explanation for this observation may be, that the recipient strains might already harbor the plasmid type of the donor and therefore not accept an additional copy. Conjugation occurred between various strains with the highest observed conjugation frequencies with the pathogenic Salmonella Typhimurium DSM 30122 and the donor E. coli 10682. In two cases, conjugation occurred very rapidly, which might become a challenge for In vivo trials.

Conclusion: Conjugation frequencies differ between donors and recipients and in time, with E. coli ESBL10682 being the donor with the highest observed conjugation frequencies. The results of this study suggest, that ESBL-carrying plasmids might spread to both commensal bacteria and pathogens in poultry and offer a basic for studies on the impact of feed additives.

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139.

Detection of toxic MCPG metabolites after ingestion of Sycamore Maple seeds in horses with Atypical Myopathy

Nachweis toxischer MCPG-Metaboliten bei Pferden mit Atypischer Myopathie nach Aufnahme von Bergahornsamen

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The consumption of fruits of *Sapindaceae*, especially ackee and litchis, well known for toxic hypoglycin A (HGA) content, can induce Jamaican Vomiting Sickness in humans with fatal outcome preferably in children (1). A lower analogue of HGA, methylenecyclopropylglycine (MCPG), was found in both seeds and arils of litschi fruits and had to be implicated as a causative agent of hypoglycemic encephalopathy in humans (1). Toxic effects of MCPG and HGA after metabolism proved by the detection of their toxic metabolites (MCPF-, methylencyclopropylformyl-; MCPA-, methylencyclopropylacetyl-) seem to be additive in humans (2). In Germany Atypical Myopathy (AM) in horses is currently only linked to HGA poisoning after seed ingestion of *Sycamore Maple* (SM, also Fam. *Sapindaceae*) with fatal outcome (3). The aetiopathology is described as an acquired enzymatic deficiency of multiple acyl-CoA dehydrogenases, a highly fatal form of non-exertional rhabdomyolysis. Indeed, until now it can´t be explained why healthy cograzers with high HGA concentrations in bodyfluids do not develop the disease (3). The aim of the study was to investigate if toxic metabolites of MCPG in body fluids of horses after SM seed ingestion are also detectable. We hypothesized that both HGA and MCPG can be measured in horses suffering from AM.

Methods: In winter 2016, 16 equines (6 warmbloods, 1 tinker, 1 noriker, 3 cold-blooded horses, 2 heavy-warmblooded horses, 3 Arabian horses, comprising 1 gelding, 12 stallions, 3 mares; 1.5 - 12 years old) with 24 h pasture turn out per day in Germany exhibited acute clinical signs of muscle pain and weakness. 14 horses died within 1 or 2 days after showing first clinical signs or were euthanized, 2 horses survived (SURV, no urine samples). In every case, AM was diagnosed by the veterinarian in charge based on the clinical signs and biochemical results. Most meaningful was the higher creatinine kinase (U/L) in deceased horses (227,183 [11,386 – 655,022] *vs* SURV horses (26,142 [3,504 – 61,080]). Urine (n = 10) and blood serum samples (n = 27) from horses collected over the timespan of disease were analyzed for HGA and MCP-compounds (UPLC-MS/MS) (2). Serum and urine from healthy horses without contact to *Acer spp.* were used as controls (CON).

Results: SM seeds were found on all pastures (69 – 1,340 µg HGA/g seed) with HGA contents similar to previous studies (3). The content of HGA in serum [nmol/L] of deceased horses ranged from 1,426 - 16,602 (SURV, 95 – 687; CON < 0.81), and in urine from 22 – 3,495 (CON < 2.4), respectively. The range of MCPA-carnitine and -glycine concentrations [nmol/L] originated from HGA metabolism in serum were 68 – 1,025 (SURV, 0.7 - 30; CON < 0.01), and 169 – 4,649 (SURV, 7 – 116; CON < 0.34) and in urine for MCPA-glycine 7,908 – 78,156 nmol/mmol creatinine. The range of MCPF-carnitine and -glycine concentrations [nmol/L] originated from MCPG metabolism in serum were 796 – 17,832 (SURV, 20 – 1,016; CON < 0.13), and 34 – 1,180 (SURV, 1.2 – 70; CON < 0.37) and in urine for MCPF-glycine 2,189 – 15,566 nmol/mmol creatinine.

Conclusion: Within the collective of AM horses, MCPG and HGA intoxication was confirmed by the presence of HGA, MCPA- and MCPF-conjugates in urine and serum. Due to these facts MCPG seems to be also apparent in SM seeds and probably can pronounce the toxic effects. Survivals were clearly below the deceased horses for all examined parameters.

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140.

Changes of free and bound cortisol in plasma and saliva during an ACTH challenge in dairy cows and horses

Veränderungen von freiem und gebundenem Cortisol im Plasma und Speichel während einer ACTH-Challenge bei Milchkühen und Pferden

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Cortisol levels reflect hypothalamic-pituitary-adrenocortical (HPA) axis activity. While most plasma cortisol is supposed to be bound to the soluble cortisol binding globulin, only free cortisol (FC) is considered the biologically active form. It must be emphasized that the objective of the present study is not a direct species comparison of dairy cows and horses. We aimed to establish a multi-species suitable method to assess FC in cows and horses which in combination with total cortisol (TC) allows interpreting proportional changes of cortisol in saliva as well as in blood in response to a standardized HPA axis activation via ACTH. We further investigated if the ratios of cortisol fractions as obtained at basal levels in healthy horses and dairy cows change during HPA axis activation, and to which extent saliva cortisol (SC) is representative for alterations in plasma FC and adrenal cortex reactivity.

Methods: Adrenocorticotropic hormone (ACTH Fragment 1-24, Sigma-Aldrich) was administered i.v. (0.16 µg/ kg BW) in 8 multiparous lactating Holstein dairy cows, whereas 5 healthy horses received a dose of 1 µg/kg BW of the synthetic ACTH1-24 (Synacthen, Novartis). Blood was collected from a jugular vein catheter before and at 30 min intervals for the following 180 min after the ACTH injection into tubes containing EDTA and the harvested plasma was stored at -20°C until analysis. Saliva samples were collected in parallel to blood samples using saliva collection tubes (Salivette). Each saliva sample was taken within 1 min after the respective blood sample and stored at -20°C after centrifugation. Total cortisol (TC) in blood was measured using a commercially available RIA (Beckman Coulter) and slightly modified for horses (1). The ratio of free cortisol (rFC) in plasma was measured using a modified ultrafiltration method as previously described (2, 3). The concentration of free cortisol (cFC) was the product of TC and rFC. Saliva cortisol (SC) in cows and horses was measured with an ELISA (Salimetrics Cortisol Enzyme Immunoassay Kit, Salimetrics). Pearson‘s correlation coefficients between SC, plasma cortisol, and cortisol fractions, as well as the ratios of SC to TC and SC to cFC at the respective time-points during the ACTH challenge were evaluated. A Bland-Altman analysis was conducted that revealed the agreement between SC and cFC. Differences in cortisol, cortisol fractions, and ratios of advanced sampling time-points (30 to 180 min after ACTH application) in relation to initial values and between time-points within horses and cows were evaluated with a MIXED model with species and time-point as fixed effects. The individual animal was considered as repeated subject. Significant effects were assumed at $P<0.05$.

Results: During the entire sampling period of the ACTH test, plasma TC was paralleled by blood cFC, rFC, and SC in both cows and horses. All cortisol fractions increased within 30 min of ACTH administration compared to basal values (0 min, $P<0.05$). Basal cFC plasma were 1.4 ± 1.0 ng/mL in horses, and 0.6 ± 0.7 ng/mL in cows. Peak TC concentration reached 63.2 ± 9.6 ng/mL and 73.2 ± 11.8 ng/mL in bovine and equine plasma, resp. Peak values of rFC averaged 17.9 ± 4.5 % in cows and 19.2 ± 7.8% in horses. Peak cFC was 11.6 ± 3.0 ng/mL in cows and 12.8 ± 3.4 ng/mL in horses. The ratio of SC to cFC in horses remained similar during the ACTH challenge suggesting that SC is recruited from plasma FC. However, SC increased less compared to plasma TC and FC during HPA axis activation in cows.

Conclusions: In conclusion, the short-term activation of the HPA axis caused not only an elevation of TC, but also a similar increase of rFC in both species. Saliva cortisol closely reflected changes of FC in horses, but less accurately in cows. The concomitant evaluation of changes among cortisol fractions might give further indications on adaptation mechanisms in glucocorticoid regulation as well as differentiate cortisol-related health disorders.

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141.

Investigations on the influence of different farrowing systems on colostrum supply and performance of piglets

Untersuchungen zum Einfluss verschiedener Abferkelsysteme auf die Kolostrumversorgung und die Entwicklung von Ferkeln

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Especially after birth, the colostrum supply of newborn piglets is of particular importance for the survival and further development of the piglets (1). The aim of this study was to investigate in what way the farrowing system and thus the locomotion influences the colostrum supply of piglets.

Methods: In five consecutive trials a total of 77 sows were stabled in three different housing systems, one restricted the sows in conventional farrowing crates, the other two loose farrowing systems allowed the sows to move freely. Therefore, a total of 26 sows assigned randomly to the commercial farrowing crates (system I), 24 sows were housed to a crate without fixation (system II) and 27 sows were assigned to a group farrowing pen system with six individual pens without fixation and a joint area (system III). During the period of farrowing, all sows in system III were confined into their individual pens. The sows with the highest parity were assigned to system II (3.13±2.35; system I: 2.56±1.63; system III: 2.33±1.21). Feed intake was measured daily. Blood samples of two heavy and light piglets per litter were taken within the first two days after birth to determine the immunocrit ratio according to the method first described by VALLET (2). Blood was collected from the same piglets again before weaning. Differences between the groups were tested by using the t-test (normal distributed) and the one way ANOVA-test (normal distributed; significance level: $p<0.05$).

Results: The sows in system I had an averaged number of born alive piglets at the point of blood sample p.n. of 14.56±4.48 (system II: 14.54±4.55; system III: 16.67±4.07). The level of the immunocrit ratio of the piglets in system I was post natum (Table 1) as well as before weaning (system I: 0.050±0.013; system II: 0.047±0.015; system III: 0.044±0.016) markedly higher than the immunocrit ratio of the piglets in system II + III. Especially the immunocrit ratio of the light piglets p.n. in system III was obviously lower than in system I + II. In analysed blood samples from piglets that didnt survive the lactation period (system I: n=4; system II: n=6; system III n=9) the immunocrit ratio was significantly lower in system III than in the other two systems (system I 0.164±0.03; system II 0.137±0.03; system III 0.116±0.04). Furthermore, the highest total litter weight of all sows after four weeks of lactation was found in system I (system I: 77.5±12.8 kg; system II: 71.3±17.0 kg; system III: 66.0±12.7 kg).

Table 1: Body weight (kg) and immunocrit of piglets, which were tested, within 48h post natum (p.n.) divided into light (< 1.4 kg) and heavy piglets (> 1.4 kg)

	Sows	Piglets	Body weight			Immunocrit		
	[n]	Σ/light/heavy [n]	p.n. Ø [kg]	p.n. light [kg]	p.n. heavy [kg]	p.n. Ø	p.n. light	p.n. heavy
System I	26	109/47/62	1.50^{A} ±0.402	1.10^{bB} ±0.086	1.80^{aA} ±0.255	0.158^{A} ±0.031	0.149^{bA}±0.034	0.164^{aA}±0.027
System II	24	97/36/61	1.58^{A} ±0.392	1.16^{bA} ±0.106	1.82^{aA} ±0.274	0.156^{A} ±0.036	0.146^{aA}±0.037	0.162^{aA}±0.034
System III	27	108/53/55	1.53^{A} ±0.425	1.15^{bA} ±0.110	1.88^{aA} ±0.286	0.133^{B} ±0.039	0.120^{bB}±0.037	0.147^{aB}±0.037

a, b ,averages differ significantly within a row between light and heavy piglets ($p < 0.05$); A, B averages differ between a column ($p < 0.05$)

Conclusion: The significant lower immunocrit of the light piglets of system III p.n. could indicate a problem with the colostrum supply especially in this system. Thus, the piglets in system III also had the lowest immunocrit ratio before weaning. In future it has to be clarified whether the immunocrit ratios will be equal in case of a larger number of samples including only sows with the same total litter weight and parity. In this event it has to be investigated in greater details if there is a problem with the colostrum transfer or the sows have insufficient colostrum production.

The project is supported by funds of the Federal Office for Agriculture and Food (BLE).

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142.

Effects of dose of pancreatic enzymes and extracorporal predigestion of the diet on prececal digestibility of nutrients – study using ileo-cecal fistulated minipigs with exocrine pancreatic insufficiency as a model for canine patients

Effekte der Enzymsubstitution und der extracorporalen Vorverdauung des Futters auf die praecaecale Verdaulichkeit der Nährstoffe – Nutzung ileo-caecal fistulierter Minipigs mit exokriner Pankreasinsuffizienz als Modelltiere für canine Patienten

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Introduction: Exocrine pancreatic insufficiency (EPI) is a common disease in dogs resulting in a distinct maldigestion and malabsorption of diverse nutrients. Pancreatic enzyme replacement therapy (PERT) is standard to treat EPI as it is considered to be safe. Because costs of PERT are relatively high measures to improve efficacy of PERT are of great interest. As no canine animal model with isolated EPI is available to study prececal (pc) digestibility (dig) of nutrients, it seems appropriate to use a porcine model [1;2]. A former study [3] using a very high enzyme dosage (300.000 IU lipase/meal) indicated only slight effects of extracorporal predigestion (ExPD) on pc dig. ExPD numerically improved pc dig of crude protein (cp) but did not cause a significant effect. It was speculated that effects of ExPD might be larger when enzyme dosage is lower. This study aimed to test, whether ExPD of feed by PERT is more relevant when a lower enzyme dosage is used.
Materials and Methods: 8 adult female minipigs, fitted with an ileo-cecal re-entrant fistula to determine pc dig of nutrients were used in this study. In 4 pigs (PL-pigs) the pancreatic duct was ligated to induce an EPI. The dry pet food contained (on dry matter basis): 28 % cp, 33 % crude fat (cfa), 17 % starch. Tests were performed as a Screening-test [4]. PERT was given at two dosages (very high dosage vs. high dosage: 300,000 resp. 100,000 IU lipase, 17,300 resp. 5767 IU protease and 306,000 resp. 102,000 IU amylase; per meal). PL-pigs received the diet five times in a randomized order: without PERT, with PERT (high and low dosage) added shortly before diet was fed; for ExPD PERT was added 10 hours before feeding and feed was stored at room temperature (20°C). The diet was always soaked for 10 hours (250 g of diet + 800 ml H2O).
Results: The pc dig of all tested nutrients was quite high in control pigs and significantly reduced in PL-pigs receiving no PERT (although pc starch dig reached 93 %). Pc dig of nutrients increased significantly in PL with PERT – reaching level of controls for cfa (for both dosages) and for cp (at higher dosage). For prc dig of cp there was also a marked effect of PERT dosage (see table 1). ExPD of food numerically increased pc dig of cp in both dosages tested.
Table 1: Prececal digestibility (%) of nutrients in healthy controls as well as in PL-pigs (without or with PERT [high dosage: 100.000 and very high dosage: 300.000 IU lipase] with or without 10 hrs of ExPD); A;B: sign. difference ($p<0.05$) between controls and PL (t-test), a,b,c: sign. difference ($p<0.05$) between differently treated PL-pigs (paired t-test)
Discussion: As studies regarding pc dig in dogs are scarce (due to public concerns) it seems appropriate to use the PL-pig model to study effects of PERT on pc dig of diets used for dogs to gain information on potential benefits of ExPD. Interestingly a „normalisation" of pc cfa dig could be achieved even when the lower dosage was used – while pc cp dig reached levels of controls only when the very high dosage was used. ExPD tended to improve pc dig of cp in PL-pigs. Due to high variation in PL-pigs these effects reached no significance but it seems noteworthy that individual variation was much higher in PL-pigs than in controls. It can be speculated that ExPD is more efficient at temperature of 38 °C, but this needs further investigations.
Conclusion: Although effect of ExPD did not reach significance, the increase of pc cp dig is noteworthy. It is noteworthy that effect of ExPD varies individually – and might be highest in individuals with a more challenging gastric milieu to substituted pancreatic enzymes. Therefore, ExPD seems worth trying on individual basis.

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143.

Feed intake patterns of fattening pigs exposed to short term disturbances in stable routine

Futteraufnahmemuster von Mastschweinen bei kurzfristigen Störungen der Stallroutine

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In practice, assessment of feed intake behaviour of fattening pigs is limited to daily feed intake calculated from feed quantities supplied to the respective barn. Individual data, however, are scare. Furthermore, studies with high time-resolution of feeding behaviour were often conducted under laboratory-like conditions involving comparably small numbers of animals per group. Here we present individual feeding patterns of fattening pigs derived from an automatic feeder. Animals were reared under practical conditions and short term disturbances typically occuring in stable routine were included to mimic environmental factors possibly modulating feed intake behaviour of animals.

Material and Methods: 96 weaned piglets were distributed to 8 pens (n=12) considering litter, sex (48:48), and body weight. Pen construction and stable routine reflected practical standard. Each pen was equipped with an automatic feeder recording individual visits, time of entering the feeder, and feed quantity consumed. During the course of fattening 2 pens each were submitted to the following disturbances in stable routine at experimental day 30 and 51/52: (1) no disturbances (control), (2) feed restriction, (3) feed confusion, and (4) water restriction and animal rotation. Animals were fed ad libitum typical grower/finisher diets after an adaption period of seven days. Initial body weight accounted for 40.0±0.6 kg (day 0). Animals were fattened with grower I feed to day 36 and then with a grower II diet until day 63. After that a finisher diet was fed until slaughter (ca. 120 kg). Individual feed intake data were recorded only until day 63 because first animals soon afterwards were removed from the pens for slaughter. For further analysis the visits > 5 g feed intake were sorted day-wise for ascending feed consumption within treatment. These visits were cumulated and the block accounting for 95-100 % of feed intake per treatment was identified in order to select daily top feed intake events. Statistical analysis included ANOVA with type of disturbance as factor.

Results and discussion: Disturbances in stable routine did not affect zootechnical or slaughter performance of animals (data not shown). Total visits per day and average feed intake per visit as well as visit characteristics explaining the top 5 % of daily feed consumption differed between treatment groups ($p<0.001$). However, this was present the entire time course of the study irrespective of the occuurence of disturbances in stable routine. Average feed quantity of top feed intake events exceeded by far the 5 % margin of average feed intake per animal and day. This suggests major day-to-day fluctuations of feed intake patterns within and between animals.

day			(1)	(2)	(3)	(4)	mean	SEM	p<
total visits and feed intake per visit (data expressed per animal), the letters indicate statistically different means									
1-35	counts	n/d	14.1b	14.5b	13.2c	17.4a	14.8	0.8	0.01
36-63	counts	n/d	14.2c	15.9b	11.5d	17.2a	14.7	0.8	0.01
1-35	feed/visit	g	138a	122bc	127b	117c	126	32	0.01
36-63	feed/visit	g	170b	155c	211a	159c	174	38	0.01
visits explaining top 5 % of total daily feed intake (data expressed per animal), the letters indicate statistically different means									
1-35	counts	n/d	1.15	1.13	1.08	1.11	1.09	0.01	0.15
36-63	counts	n/d	1.07b	1.23a	1.17ab	1.14ab	1.15	0.01	0.01
1-35	feed/visit	g	521b	544a	547a	512b	530	4	0.01
36-63	feed/visit	g	623c	643b	804a	653b	676	3	0.01

Conclusion: Feed intake patterns of fattening pigs seem to persist within an animal group once they have been established. Patterns include events with ample feed intake that are not regularly occurring on a day-to-day basis. Disturbances in stable routine happening occasionally do not seem to modulate feed intake patterns of fattening pigs or overall zootechnical and slaughter performance.

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144.

Trade-off between parasite resistance and tolerance in a high and a lower performing layer chicken genotype: short-term response to experimental nematode infections

Einfluss des Leistungsgenotyps von Legehennen auf Resistenz und Toleranz gegen eine Parasiteninfektion in der frühen Legeperiode

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Nematode infections in layer chickens, particularly in organic and free-range systems, are highly prevalent, implying potential welfare problems and economic losses due to parasitism. Strategies that the host animal uses to deal with nematode infections may closely be related with their performance levels determined by their genetic make-up. Since layer chickens have not been selected directly for nematode resistance, but high performance and efficiency, comparison of genotypes with different performance levels may help better understand whether and how resistance (ability to lower pathogen burden) and tolerance (ability to perform well despite infections) are traded off at the time of challenge, particularly under high metabolic load. Therefore, two chicken genotypes varying markedly in their laying performance were compared in terms of their short-term responses to nematode infections.

Methods: In total, 180 laying hens of a high performing (Lohmann Brown plus, **LB**) and a lower performing genotype (Lohmann Dual, **LD**) were either infected at peak production (24 week of age) with 1,000 eggs Ascaridia galli and Heterakis gallinarum or were kept as uninfected controls. Infected hens were necropsied at timed intervals up to 6 weeks post infection (wpi) to determine their infection intensities with both nematodes. Individual worm burdens, plasma and egg-yolk concentrations of a worm-specific immunoglobulin Y (**IgY**), and egg quality traits (e.g. weight, color and thickness of shell and yolks, Haugh unit) as well as egg-yolk fatty acids (**FA**) composition were quantified at wpi 2, 4 and 6. Hens were fed on a commercial layer diet ad libitum. Laying performance, egg weight and feed intake were determined at pen levels at weekly intervals. Data were analyzed with a 3-way-ANOVA to account for the effects of infections, genotype, wpi and interactions using SAS (V9.4).

Results: Individual worm burdens with either nematode species did not differ between the two genotypes at any time point ($P>0.05$). Nematode infections did not affect external egg quality traits ($P>0.05$). Infected animals of both genotypes showed elevated levels of IgY in both blood plasma and egg yolks ($P<0.001$). Moreover, infected LB hens had higher ($P<0.05$) IgY levels in plasma at wpi 3 as compared with infected LD hens, whereas there was no difference between these two groups in egg-yolk IgY levels ($P>0.05$). Laying performance of LD hens (86.8%) was not influenced by the infection ($P>0.05$), whereas LB hens showed impaired performance (86.7 vs. 94.9%) due to infection ($P<0.05$), which was accompanied by lower ($P<0.05$) feed intake (112 vs. 122 g/day). Egg weight was higher ($P<0.001$) in LB than in LD, and remained unaffected by the infection ($P>0.05$). Infection impaired feed conversion efficiency (2.20 vs. 2.08 g feed:egg) in both genotypes ($P<0.05$). Yolk-weight was similar for both genotypes ($P>0.05$), and was not influenced by infection or time effects ($P>0.05$). Infection reduced ($P<0.05$) both fat content (5.2 vs. 5.9%) and the proportion of polyunsaturated FA (**PUFA**) in the egg yolks.

Conclusions: The high performing genotype is also able to resist its natural pathogens as the lower performing one, whilst penalizing its performance. This indicates prioritization of resistance over tolerance under high metabolic burden (i.e. peak production). Laying performance of the lower performing genotype was not affected by infection, likely indicating a more balanced trade-off between resistance and tolerance. Infections had no effect on external egg quality traits, but on certain functional yolk nutrients (e.g. PUFA). Lowered fat content and proportion of PUFA due to infection in both genotypes might be indicative of an impaired reproductive fitness, as they serve as the major source of energy and essential FA to the chick embryo, respectively. It is concluded that the high performing genotype is not necessarily less resistant, but less tolerant to the short-term effects of nematode infections.

Funded by the German Government´s Special Purpose Fund held at Landwirtschaftliche Rentenbank.

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145.

Intake potential by cows of different woody and herbaceous plants rich in phenols

Verzehrseigenschaften verschiedener phenolreicher Holzgewächse und krautiger Pflanzen bei Kühen

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Plant secondary compounds (PSC) play an important role in developing feeding strategies for ruminants with the aim to reduce methane emissions and to promote digestion processes. However, high levels of PSC can reduce the palatability of the feed and concomitantly feed intake and productivity of the animals are compromised. This explains why the mitigation of methane emission from ruminal fermentation with promising plant species are often less successful *In vivo* than *In vitro*. The aim of the present study was, therefore, to quantify the palatability of feeding six plants as supplements to the ration of dairy cows. The plants had been selected based on their potential to reduce methane and ammonia formation at maintained *In vitro* digestibility in a preceding *In vitro* study (1).

Methods: The following materials were tested: Leaves from silver birch (*Betula pendula*), hazel *(Corylus avellana*), wood avens (*Geum urbanum*), blackcurrant (*Ribes nigrum*), and green grape vine (*Vitis vinifera*) as well as the herb from rosebay willow (*Epilobium angustifolium*). The ground plants were combined with lucerne in different proportions (54 to 98%) to achieve the same total phenol (TP) content of 4.1% in dry matter. These materials were pelleted and provided separately from a mixed ration (consisting of grass silage, maize silage, hay and concentrate) in a ratio of 0.4:0.6. The experiment was conducted with six late-lactating Brown Swiss cows covering a range from first to fifth lactation. The rations were fed separately three times a day. During the 7 days of adaptation period lucerne-only pellets were fed (control) beside the mixed ration. Thereafter, the cows were offered the pellets including the different plants. All six types of plant pellet were fed in randomized order to each cow for a duration of 3 days each. The intake of the mixed ration and the pellets were recorded daily. The intake of control pellets during the adaptation period were used as reference for well palatable pellets with a low TP concentration. The Palatability Index (PAL) was calculated for the test plant pellets as follows: PAL (%) = (pellet intake (kg) / pellet offered (kg)) / (lucerne pellet intake (kg) / lucerne pellet offered (kg)) × 100 (2). The data were analysed 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 crude protein content varied from 14.9 (rosebay willow) to 19.8% (lucerne-only), that of neutral detergent fibre ranged from 29.6% (blackcurrant) to 43.2% (lucerne-only). The lignin content of the pellets supplemented with birch and blackcurrant was twice as high as those containing rosebay willow. The actually measured TP content of the pellets ranged from 4.8% (hazel) to 9.5% (rosebay willow). The mixed ration was completely consumed by all animals independent of the type of pellet fed. Therefore, the PAL index could be calculated based exclusively on the intake of the lucerne-only pellets during the adaptation period. The palatability of pellets with hazel, rosebay willow, avens and grape vine was higher ($p<0.01$) than those with birch and blackcurrant.

Conclusion: The hazel pellets with the lowest TP concentration were the most palatable test plant pellets in the present study. Unexpectedly, the rosebay willow pellets with the highest TP concentration were not the least palatable ones. The results suggest instead that lignin, highest in content in blackcurrant and birch, was most adverse for palatability. In a subsequent experiment with dairy cows, the potential of hazel leaves to mitigate methane and urinary nitrogen emissions will be examined.

This study was supported by the ETH Zurich Research Grant (ETH-49 15-1) and by the China Scholarship Council.

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146.

Enhancing health-beneficial fatty acids in milk fat of early lactating cows by high-quality hay

Qualitativ hochwertiges Heu erhöht den Anteil an gesunden Fettsäuren im Milchfett von Kühen in der Frühlaktation

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High-quality (HQ) hay is characterized by its richness in energy and protein and therefore could be used as an alternative to grains in the feeding of dairy cows. Grass and hay are generally rich in omega-3 fatty acids (FA), thus feeding HQ hay might be beneficial not only by improving rumen health and reducing the competition over human edible foods but also by improving the health-promoting omega-3 FA in milk. However, effects of HQ hay on milk FA are underexplored. Besides, the extent of increased body fat mobilization during early lactation might disguise dietary effects on milk FA. The present study aimed at finding that, in terms of milk FA profile and milk yield, to which extent the HQ hay can satisfactorily substitute concentrate in early lactating dairy cows.

Methods: Twenty four Simmental cows were balanced by parity and previous milk yield and assigned to 4 different diets (n = 6 per diet) at 10 days before expected calving date until 28 days thereafter. The test diets were one control diet (CON) containing 60% fiber-rich hay (4.6 MJ NE_L, 65 g CP, 110 g sugar and 646 g NDF per kg DM) plus 40% concentrate and three diets containing HQ hay (60HQ, 75HQ, and 100HQ) with graded amounts of concentrates (40%, 25% and 0% of DM, respectively). Besides the energy, protein and sugar content (6.9 MJ, 223 g, and 198 g per kg DM, respectively), the HQ hay was also richer in total FA (2% of forage DM) dominated by 18:3 n-3 (56% of total FA) and 18:2 n-6 (18%) compared to the regular hay (0.5% total FA, thereof 32% as 18:3 n-3 and 21% as 18:2 n-6). Data of the present study included DM and FA intake, milk yield, milk FA yield, and the FA profile of the milk taken on d7, 14, 21 and 28 postpartum. Data were analyzed as repeated measure using Proc Mixed of SAS to obtain the effects of diet, days and their interaction while cow and parity were considered as random effects in the model.

Results: There was no diet and day interaction on the variables studied, except for n-6:n-3 ratio ($P<0.001$). On average of the days, 100HQ (17.5 kg) had lower DMI than the other diets (20.3-20.9 kg). Milk yield was higher in 60HQ (38 kg) than the other diets (32-33 kg). Milk FA yield ranged from 1.1 – 1.6 ± 0.16 kg/d ($P = 0.146$). The intake of 18:2 n-6 was higher with 60HQ and 75HQ (80 g/d) than with CON (64 g/d) and 100HQ (59 g/d). The 18:3 n-3 intake was greater with the HQ diets (121, 171 and 187 g/d with 60HQ, 75HQ and 100 HQ, respectively) than with CON (33 g/d). The proportion of milk saturated FA, representing the de novo sources, was equal between CON and 100HQ and was lower than in 60HQ and 75HQ. The opposite result was found for the proportions of 18:1 n-9 and total monounsaturated FA which are associated with body fat mobilization. Milk FA deriving from the rumen sources including odd and branch-chain FA and biohydrogenation products (18:0 and cis/trans isomers of 18:1 and 18:2) increased with increasing HQ hay level in the diet. For preformed FA, the proportion of milk 18:2 n-6 was higher with CON (2.05% of total FA) compared to the HQ diets (1.58 – 1.73%), whereas the proportion of 18:3 n-3 increased with increasing level of HQ hay (0.54, 0.68, 0.95 and 1.04% in CON, 60HQ, 75HQ, and 100HQ, respectively). Thus, the n-6:n-3 ratio decreased with increasing level of HQ hay, being the highest in CON (3.85) and lowest in 100HQ (1.71). On average across the diets, intake of DM and FA and milk yield increased with day after calving while the milk FA yield remained unchanged (1.3 - 1.4 kg/d). The proportions of de novo FA and odd and branch-chain FA increased with day after calving, whereas the other grouped FA and individual FA studied decreased with day after calving.

Conclusions: Despite the reduced intake, solely feeding of the HQ hay sustained the milk yield and beneficially modulated the milk FA profile towards more omega-3 FA without differences in milk FA of the endogenous origin compared to the CON diet with 40% concentrate. Feeding the HQ hay plus 40% concentrate was superior in improving milk yield.

We thank S. Sharma for her excellent contribution to the sample analysis. This study was supported by the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management.

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Effects of abomasal infusion of essential fatty acids and conjugated linoleic acid on hepatic energy metabolism including the somatotropic axis of dairy cows fed a ration with reduced n-3 fatty acid content

Einfluss einer abomasalen Infusion von essentiellen Fettsäuren und konjugierter Linolsäure auf den Energiestoffwechsel in der Leber, einschließlich der somatotropen Achse, bei Milchkühen mit einer reduzierten Versorgung mit n-3 Fettsäuren im Grundfutter

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Common diets in dairy production are mainly based on corn silage (CS), and provide low amounts 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 ALA and CLA status in lactating cows (1). In our first results of the present study we showed an increase of respective supplemented fatty acids in plasma and milk fat when replenishing ALA and CLA by abomasal infusion (2). The aim of the present study was to investigate the effects of ALA and CLA replenishment on hepatic energy metabolism including the somatotropic axis of lactating dairy cows fed a CS based diet.

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. 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 *t10, c12*, 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. Plasma concentrations of metabolites (glucose, non-esterified fatty acids [NEFA], triglycerides, cholesterol) and hormones (insulin, insulin-like growth factor [IGF]-I, IGF binding proteins [IGFBP]-2-4) related to energy metabolism were analysed at wk 0, 2, 4 and 6 of each treatment period. Liver biopsy samples were collected before the start of the trial and at wk 6 of each treatment period and expression of genes related to the somatotropic axis (liver specific growth hormone receptor [*GHR1A*], *IGF1,* IGF1 receptor [*IGF1R*] and *IGFBP2* and *3*) as well as genes related to regulation of cholesterol and lipid metabolism (3-hydroxy-3-methyl-glutaryl-CoA synthase 1 [*HMGCS1*], sterol regulatory element-binding factor 1 [*SREBF1*], peroxisome proliferator-activated receptor α and γ) were measured by quantitative real-time PCR. Data were analysed by repeated measurement ANOVA using the MIXED procedure of SAS containing treatment, dosage, and its interaction as fixed effects and week in milk as covariate. For data of relative hepatic mRNA expression, the factor dosage was excluded.

Results: Plasma concentration of IGF-I increased in CLA and was higher at the beginning of the treatment period in CTRL and EFA than in CLA ($P<0.01$). Hepatic mRNA expression of *GHR1A* tended to be higher ($P<0.1$) in EFA+CLA than in CTRL, whereas expression of *IGF1* and *IGF1R* was similar in all groups. The hepatic expression of *IGFBP2* showed no significant differences, but same treatment pattern as measured for plasma IGFBP-2 concentration with lower ($P<0.05$) concentration in EFA+CLA than in CTRL. Hepatic gene expression of *HMGCS1* was upregulated in all groups, but was highest in EFA+CLA ($P<0.05$). Plasma concentration of cholesterol increased in all groups ($P<0.05$), except CLA, reaching highest levels in EFA+CLA and CTRL ($P<0.05$). Expression of *SREBF1* tended to be lowest in EFA and EFA+CLA ($P=0.05$).

Conclusions: Supplementation of EFA and CLA affected the cholesterol biosynthesis with regard to increased hepatic expression of *HMGCS1* and elevated cholesterol plasma levels. EFA treatments led to a downregulation of the expression of *SREBF1* in liver tissue, pointing at an influence on the regulation of lipid and cholesterol metabolism. Increasing plasma IGF-I in CLA and lower plasma IGFBP-2 and greater hepatic *GHR1A* gene expression in EFA+CLA were indicating an improved energy status in both CLA treated groups and supported previous findings of an improved energy balance in CLA cows (2).

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148.

T and B cell subsets in neonatal calves depending on maternal conjugated linoleic acid and essential fatty acid supply

T-und B-Zellverteilung in neugeborenen Kälbern in Abhängigkeit von der maternalen Versorgung mit konjugierter Linolsäure und essentiellen Fettsäuren

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The generation of an own strong immune system after birth is a key factor in the development of robust calves. Especially the development of the intestinal immune system is important because of its key role in the defense against pathogens but also in the adsorption of essential nutrients. Previous studies give evidence that the maternal supply with essential fatty acids (EFA) or conjugated linoleic acid (CLA) can improve the functionality and proliferation capability of cellular components of the immune system of calves (1; 2). A potential impact of EFA and CLA on the intestinal immune system might also affect the defense of this local immune system. Therefore, the present study focused on the influence of maternal CLA and EFA supply on intestinal T cell subsets in neonatal calves.

Methods: A total of 6 German Holstein cows were abomasally supplied either with CLA (Lutalin™ = feed additive, 25 % *cis*-9, *trans*-11 CLA + 25 % *trans*-10, *cis*-12 CLA) or a combination of CLA and EFA (Lutalin™ + Linseed oil + safflower oil) from week 9 before calving and during following lactation period. Three additional cows acting as control group received coconut oil for the same period (3). The 9 calves (4 male calves, 5 female calves) born from mentioned dams and fed with their milk were slaughtered 5 days after birth. Tissues from mid jejunum, terminal ileum and jejunal Peyer´s patches (PP) were dissected and contained leucocytes were isolated by modified methods of Liermann et al. (4). The portions of CD2-; CD4-; CD8- and CD21-positive cells and T and B cell subsets were quantified by flow cytometry. For statistical analysis MIXED procedure of SAS Enterprise Guide 6.1 was used. The statistical model included the fixed effects maternal supplementation and sex. At $p < 0.05$ differences were declared as significant.

Results: The portion CD2+ T cells from jejunal lamina propria (LP) in calves of dams supplied with CLA were significantly decreased compared to calves of dams from control group or dams with CLA+EFA supply ($p < 0.05$). Furthermore, CD2+ T cells were higher frequented in jejunal LP of female calves than in jejunal LP of male calves ($p < 0.01$). There were no effect of maternal supply on CD4+ T cell subsets ($p > 0.05$), however, CD4+ T cell subsets were significantly lower in case of jejunal intraepithelial leucocytes and in jejunal LP in female calves compared to CD4+ T cell subsets in similar localisations in male calves ($p < 0.05$). CD8+ T cell subsets isolated from jejunal epithelium were significantly decreased by maternal CLA supply compared to subsets in calves of dams received CLA+EFA ($p < 0.05$) and tended to be lower compared to subsets in calves of dams of control group ($p = 0.072$). In general, CD21+ B cell subsets in jejunal and ileal LP were increased by maternal CLA supply ($p < 0.010$). Further, CD21+ B cell subsets were higher in jejunal LP of male calves compared to subsets of female calves ($p < 0.001$). Also significant interaction between maternal supply and sex were detected in case of CD21+ cells isolated from jejunal LP ($p < 0.001$) which was mainly related to the fact that one male calve from the CLA group had higher CD21+ cell subsets than all other animals. T and B cell subsets of PP were not influenced by maternal supply or sex ($p > 0.05$). Interestingly, in contrast to calves of the CLA group T and B cell subsets of calves from dams supplied with CLA+EFA were similar to subsets in calves from control cows in all localisations.

Conclusion: The current study clearly shows an impact of maternal supply with CLA on the intestinal immune system of neonatal calves. However, this effect seemed to be offset by a combined supply with EFA.

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149.

Studies on chemokine receptor CXCR4 expression by bovine leukocytes and the effects of essential fatty acid supplementation in dairy cows fed a maize based ration

Studien zur Expression des Chemokinrezeptors CXCR4 durch bovine Leukozyten sowie Effekte einer Supplementierung mit essentiellen Fettsäuren bei Milchkühen, welche mit einer auf Mais basierenden Ration gefüttert wurden

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The chemokine receptor CXCR4 is involved in cell migration and cell adhesion as well as extravasation, thereby functioning as a pro-inflammatory receptor after activation by its ligand cell-derived factor 1 (SDF-1/ CXCL12) (1). In bovine mammary gland the receptor is upregulated by experimental E. coli mastitis (2). The abundance of CXCR4 on human monocytes is indirectly down-regulated by conjugated linolenic acids (CLA) (3), which are derived from essential fatty acids (4). Aim of the study was to analyse the distribution of CXCR4 on bovine leukocytes as well as its regulation in dairy cows, which were fed a maize based ration and supplemented with essential fatty acids and CLA.

Methods: The differential distribution of CXCR4 on bovine leukocytes was investigated by flow cytometry and qualitatively confirmed by Western blot. Fatty acid effects on CXCR4 abundance on leukocytes were researched in rumen fistulated Holstein cows (n = 4; 126 ± 4 days in milk) fitted with abomasal infusion tubes, arranged in a 4 x 4 Latin square model (5). Cows were fed a TMR based on maize silage and supplemented twice-daily with three successively rising lipid dosages. Each dosage was given for two weeks and doubled every two weeks, resulting in a six week treatment period, followed by a three week wash out phase. Supplements were coconut oil delivering medium-chain fatty acids (FA) (38 g/ d) linseed-safflower oil mix, delivering n-3 FA (EFA, 39 and 2 g/d), Lutalin® (*c9,t11* and *t10,c12* CLA, 5 g/d, respectively), and EFA + CLA. The abundance of CXCR4 expression on leukocytes and peripheral blood mononuclear cells (PBMC), the latter stimulated with phorbol myristate acetate (PMA), was evaluated in blood samples collected after each treatment and wash out period. Quantitative data were analyzed by mixed model of SAS using repeated measurement analysis with treatment as fixed effect and week in milk as covariate. LSMEANS were compared by Tukey test. Data are presented as LSMEANS ± SEM.

Results: Mainly monocytes expressed CXCR4 (84.4%), followed by lymphocytes (60.3%) and granulocytes (1.6%). Comparable CXCR4 abundance were found in protein extracts of all three cell types. Significant differences between the treatment groups or in their relation to the washout phase were not observed and accordingly data were merged. Lower CXCR4 expression density was found on leukocytes of animals supplemented with the highest dosage of all fatty acids compared with the washout phase ($P<0.05$). However, PMBC or PBMC stimulated with PMA were not affected.

Conclusions: The expression profile of CXR4 on bovine leukocytes differed from the distribution in humans (6), which needs further clarification. The handling of the cells during PBMC isolation may have affected CXCR4 expression. CXCR4 expression density on leukocytes was lower in fatty acid supplemented cows compared with cows fed solely the maize based ration. Therefore, the pro-inflammatory potential might be higher in maize fed cows. Further analyses are necessary to clarify the importance of CXCR4 expression of bovine leukocytes for the innate immune mechanisms.

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150.

Milk fatty acid composition of cows fed varying red clover to maize silage ratios in the diet

Milchfettsäuremuster von Kühen bei Fütterung verschiedener Rotklee-Maissilage-Verhältnisse in der Ration

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The aim of this study was to investigate the effect of replacing maize silage (MS) with red clover silage (RCS) on the milk fatty acid (FA) composition of cows with focus on the FA related to ruminal biohydrogenation (BH) and/or promoters of human health.

Methods: Forty-four German Holstein cows were used in a 4×4 Latin square trial with 21-d periods (13 d adaption followed by 8 d sample collection of milk). Cows averaged 38.7±7.3 kg/d milk, 1.9±1.1 lactations, and 149±103 days in milk at the start of the trial. Four experimental diets were offered as total mixed rations and consisted of a constant forage to concentrate ratio (75:25 on a DM basis) with targeted proportions of RCS to MS in diet DM of 15:60 (RCS15), 30:45 (RCS30), 45:30 (RCS45), and 60:15 (RCS60). All diets contained (on a DM basis) 9% lupine seeds and 16% soybean meal plus wheat in different ratios to obtain isonitrogenous diets. During each sampling phase, milk samples were taken from each cow once a day, alternating between morning and afternoon milking, and pooled per cow according to milk yield. Milk FA composition (g/100 g of detected FA) was determined by gas chromatography.

Results: The proportions of linoleic acid (LA, *c*9*c*12 C18:2) in the milk fat were reduced by 4%, which was accompanied by a reduction of LA content in the diet. However, apparent recovery of LA from feed into milk increased linearly ($P < 0.001$, data not shown) with incremental levels of RCS, suggesting reduced ruminal BH of LA. The proportions of vaccenic acid (VA, *t*11 C18:1) declined ($P < 0.001$), which may be linked to inhibited BH of LA. Oleic acid (OA, *c*9 C18:1) increased linearly ($P = 0.003$) in the milk fat, although the dietary concentration of OA declined, which may indicate an inhibition of the ruminal BH of OA. The proportions of α-linolenic acid (α-LNA, *c*9*c*12*c*15 18:3) in the milk fat was increased in two fold, which resulted from the linear increase in α-LNA intake with incremental levels of RCS in the diet. Rumenic acid (RA), a conjugated linoleic acid (*c*9*t*11 CLA), was decreased by 24% ($P < 0.001$), probably due to inhibited ruminal BH of LA. On the other hand, reduced RA in the milk fat was probably due to reduced availability of VA produced in the rumen, and the consequently low VA available to be desaturated to RA in the mammary gland by Δ9-desaturase. Increasing levels of RCS increased linearly the proportions of polyunsaturated FA (PUFA, $P < 0.001$) and, simultaneously, decreased linearly the proportions of saturated FA (SFA) in milk fat ($P < 0.001$), especially those of C12:0 and C14:0 (data not shown), whereas proportions of monounsaturated FA (MUFA) were unaffected by diets ($P = 0.55$).

	Diet					*P*-value		
FA (g/100 g FA)	RCS_{15}	RCS_{30}	RCS_{45}	RCS_{60}	SEM	Diet	L	Q
Vaccenic acid	0.80^{a}	0.69^{b}	0.61^{c}	0.61^{c}	0.03	<0.001	<0.001	<0.001
Oleic acid	15.4^{b}	15.7^{ab}	16.1^{ab}	16.2^{a}	0.43	0.02	0.003	0.74
Linoleic acid	2.06^{a}	2.07^{a}	2.03^{ab}	1.98^{b}	0.03	<0.001	<0.001	0.04
α-Linolenic acid	0.50^{d}	0.70^{c}	0.92^{b}	1.13^{a}	0.02	<0.001	<0.001	0.37
Rumenic acid	0.37^{a}	0.32^{b}	0.28^{c}	0.28^{c}	0.01	<0.001	<0.001	<0.001
SFA	73.2^{a}	72.9^{ab}	72.3^{ab}	72.0^{b}	0.46	0.005	<0.001	0.99
MUFA	21.9	21.9	22.2	22.2	0.46	0.55	0.18	0.98
PUFA	3.94^{d}	4.11^{c}	4.25^{b}	4.41^{a}	0.06	<0.001	<0.001	0.92

$^{a-d}$Least square means within the same row with different superscripts differ at $P < 0.05$. L = Linear effect; Q = quadratic effect.

Conclusion: Feeding RCS increased the intake of α-LNA and its proportion in milk fat of cows. However, the reduction of proportions of LA, RA and VA in milk fat outweighed the benefits. This study shows that it is possible to alter the milk FA composition in terms of PUFA and SFA by increasing the level of RCS at the expense of MS.

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151.

Effects of essential fatty acids and conjugated linoleic acid on performance and energy metabolism in dairy cows fed a diet with reduced n-3 fatty acid content during the transition period

Einfluss von essentiellen Fettsäuren und konjugierter Linolsäure auf die Leistung und den Energiestoffwechsel bei Milchkühen mit einer reduzierten Versorgung mit n-3 Fettsäuren im Grundfutter während der Transitphase

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Increasing milk yield changes the nutritional management in dairy cattle farming from pasture based feeding to systems favoring corn silage (CS) based diets, with low amounts of grass silage, resulting in a low a-linolenic acid (ALA) supply. In a preliminary study we have shown that feeding a CS based diet leads to a reduced status of ALA and conjugated linoleic acid (CLA) in lactating cows (1). The present study aims to investigate the effects of replenishing essential fatty acids (EFA) and CLA in dairy cows fed a CS based diet on performance and energy metabolism during the transition period.

Methods: Rumen fistulated German Holstein cows (n=40; 11000 kg milk/305 d in 2nd lactation) were studied in 5 blocks of 8 cows, respectively, from wk 9 antepartum (ap) to wk 8 postpartum (pp). A CS based total mixed ration was fed *ad libitum* during lactation (wk 22-6 ap and wk 0-8 pp, 6.5 MJ NEL/kg dry matter (DM)) and dry period (wk 6-0 ap, 6.0 MJ NEL/kg DM). The cows were fitted with abomasal tubes and assigned to 4 oil supplementation groups. Cows were daily supplemented from wk 9 ap until wk 8 pp either with coconut oil (CNTR, 76g/d), linseed and safflower oil (EFA, 78 and 4g/d), Lutalin® (CLA, *c9, t11* and *c10, t12* in equal amounts, 10g/d) and EFA+CLA. During dry period, each dose was halved. The improved status of respective fatty acids (ALA, CLA) in blood and milk was recently shown in a companion study (2). DM intake (DMI) and milk yield were recorded daily, milk composition, body weight (BW), body condition score (BCS) and back fat thickness (BFT) were measured once weekly. Plasma concentrations of nonesterified fatty acids (NEFA), triglycerides (TAG), cholesterol, beta-hydroxybutyrate (BHB), glucose, insulin and glucagon were measured in blood samples taken 63, 42, 35, 28, 21, 10 d ap, on d 1 pp and once weekly up to d 56 pp. Data were analysed using the MIXED procedure of SAS by repeated measurement ANOVA containing treatment, time, block and the treatment × time interaction as fixed effects, and breeding interval and milk yield during 2nd lactation as covariates.

Results: DMI increased in all groups with time pp ($P<0.001$), but tended to be lower in CLA ($P<0.1$) than in EFA (wk 7) and in CNTR (wk 8). Milk yield pp was not affected by the treatment but in late lactation milk yield declined in all groups but not EFA ($P<0.01$). CLA supplementation reduced milk fat ($P<0.001$) and energy-corrected milk in CLA and EFA+CLA ap ($P<0.001$) and pp ($P<0.01$ or less). Energy balance was improved ($P<0.001$) pp in both CLA treated groups. The reduced fat content in milk of the CLA group was associated with an elevated milk citrate concentration pp ($P<0.01$). BW, BCS and BFT decreased ($P<0.001$) in all groups pp. Glucose and BHB concentrations changed with time ($P<0.001$) and on d 21 pp plasma glucose was higher ($P<0.05$) in CLA than in EFA. After calving, plasma concentration of NEFA increased in all groups ($P<0.001$) and was higher ($P<0.05$) in CNTR (d 21 and 28) and EFA (d 21) than in CLA and EFA+CLA. Plasma TAG decreased ($P<0.001$) around calving in all groups and was higher ($P=0.05$) on d 28 pp in CNTR than CLA. Concentration of cholesterol decreased ($P<0.001$) after drying off and rose pp in all groups ($P<0.001$), but in EFA+CLA tended to increase more steeply than in the other groups ($P<0.1$) and tended to be higher ($P=0.1$) on d 56 pp in EFA+CLA than in EFA. Plasma insulin concentration decreased ($P<0.001$) pp in all groups, was highest on d 1 pp in CLA ($P<0.05$) and was higher during the transition period in CLA than CNTR ($P<0.05$).

Conclusions: Our data confirmed the reduced milk fat content and improved energy status in cows treated with CLA. Elevated milk citrate might point at a reduced NADPH synthesis for fatty acid synthesis in the mammary gland of the CLA group. Metabolic and endocrine changes in blood plasma support the improved energy status in CLA cows and suggest an important function of CLA on the energy balance pp in cows.

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152.

Influence of essential fatty acids and conjugated linoleic acid on acute phase response, antioxidative status , retinol and α- tocopherol concentration in blood plasma of dairy cows fed a diet with reduced n-3 fatty acid content during the transition period

Einfluss von essentiellen Fettsäuren und konjugierter Linolsäure auf die Akut-Phase-Reaktion, den antioxidativen Status sowie die Retinol- und α-Tocopherol-Konzentration im Blutplasma von Milchkühen mit reduziertem Gehalt an n-3 Fettsäuren in der Grundration während der Transitphase

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One main natural source of n-3 essential fatty acids (EFA) and conjugated linoleic acid (CLA) for cows is pasture or cut-and-carry grass, which are nowadays reduced in the ration of dairy cows due to the replacement of fresh feeds by preserved diets such as corn-silage (CS) and starch from concentrate. The most important EFA for ruminants are linolenic acid (n-3) and linoleic acid (n-6) which, to a large extend, are turned into CLA by biohydrogenation (1). Both EFA and CLA are known for their positive impact on health and immune status in several species (1). An imbalanced or reduced EFA and CLA supply may contribute to an inadequate inflammatory and immune response of dairy cows. The present study reveals the influence of EFA and CLA supply on acute phase response (APP), antioxidative status and retinol and α- tocopherol concentrations in blood plasma of dairy cows fed a CS based diet with a low fat content (especially n-3 EFA; 2) during the transition period.

Methods: Forty lactating rumen-fistulated Holstein-Friesian cows (11000 kg milk/305 d in 2nd lactation) were investigated in 5 blocks of 8 cows, respectively, and received a CS based total mixed ration during lactation (wk 22-6 before and wk 0-8 after calving, 6.5 MJ NEL/kg dry matter (DM)) and dry period (wk 6-0 before calving, 6.0 MJ NEL/kg DM) (3). The diet was fed *ad libitum*. From wk 9 antepartum (ap) until wk 8 postpartum (pp) cows were supplied twice daily by abomasal infusion either with coconut oil (CNTR, 76g/d), linseed and safflower oil (EFA, 78 and 4g/d), Lutalin® (CLA, *c9, t11* and *c10, t12* in equal amounts, 10g/d) or a combination of EFA+CLA (3). The dose was halved during dry period. Blood samples were taken 63, 42, 35, 28, 21, 10 d before expected calving, on d 1 after calving and thereafter weekly up to 56 d to measure plasma concentrations of haptoglobin, interleukin (IL) 6 and 1β, paraoxonase, oxygen radical absorbance capacity (ORAC), ferric reducing ability of plasma (FRAP), retinol, α-tocopherol and β-carotene to evaluate immune response and antioxidative status. Data were analysed by MIXED procedure of SAS, repeated measurement ANOVA with treatment, time, block and treatment × time interaction as fixed effects and breeding interval and milk yield from 2nd lactation as covariates.

Results: Plasma haptoglobin concentration peaked around calving in all groups ($P<0.001$) but the pp decline tended to be delayed in EFA ($P<0.1$) and concentrations were lower ($P<0.05$) on d 35 in EFA than in CNTR. IL6 and IL1β generally decreased ($P<0.001$) pp and IL1β tended to be highest ($P<0.1$) in EFA at the end of the study. Plasma ORAC concentration showed variable time changes ($P<0.001$) throughout the trial and tended to be lower ($P<0.1$) in CLA than CNTR. Plasma concentrations of FRAP increased ($P<0.001$) and of paraoxonase declined ($P<0.001$) during calving; the increase of paraoxonase pp was greater ($P<0.05$) in EFA+CLA than in CNTR. Plasma retinol, α-tocopherol and β-carotene concentrations declined towards calving and then continuously increased ($P<0.001$), but the increase of α-tocopherol was lowest for EFA (interaction $P<0.05$). Plasma α-tocopherol was lower ($P<0.05$) on d 1 pp in CLA than in CNTR and plasma retinol was higher ($P<0.05$) on d 21 pp in CLA than EFA+CLA.

Conclusions: APP and antioxidative status showed typical time changes during calving, but EFA and CLA treatment showed only minor effects on the respective time pattern. Time pattern of fat-soluble vitamins indicated an enhanced utilisation of these vitamins around calving and the EFA and CLA treatment effects on fat-soluble vitamins warrant further investigation.

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153.

Impact of the maternal supplementation with essential fatty acids and conjugated linoleic acid on the metabolism of neonatal calves: First results

Einfluss der maternalen Supplementation mit essentiellen Fettsäuren und konjugierter Linolsäure auf den Stoffwechsel neugeborener Kälber: Erste Ergebnisse

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In modern dairy nutrition, pasture is commonly replaced by corn silage based rations resulting in an increased intake of linoleic acid at the expense of α-linolenic acid (ALA). During gestation, a changed maternal intake of these essential fatty acids (EFA) and conjugated linoleic acid (CLA) as a natural ruminal intermediate of biohydrodenation affects also the availability of fatty acids for the foetal calf (1, 2). However, the consequences of a changed maternal fatty acid supply for the calf are still unclear. Therefore, the present study aims to investigate the effect of a variable maternal supply with EFA, especially ALA, and CLA on metabolic parameters in plasma of neonatal calves.

Methods: A total of 38 calves were studied in 5 blocks of 7 – 8 calves, respectively, during the first 5 d of life. All calves were delivered by dams abomasally supplemented either with coconut oil (CTRL), linseed and safflower oil (EFA), Lutalin® (CLA) or a combination of EFA and CLA (EFA+CLA) during the last 9 wk of gestation and the following lactation (3). During the experimental period, each calf was fed with colostrum from its own dam. Concentrations of fatty acids were measured in colostrum and in blood plasma on d 1 and 5. Blood samples for measurement of β-hydroxybutyrate (BHB), glucose, lactate, non-esterified fatty acids (NEFA), triglycerides, cholesterol (total and in high [HDL-C] and low density lipoprotein [LDL-C]), insulin and glucagon were taken daily before morning feeding from d 1 to d 5 and 2 h after feeding on d 5. On d 4, blood samples were collected for 10 h after feeding. Data analysis was conducted by means of the MIXED procedure of SAS as repeated measurement ANOVA containing treatment, time (and their interaction), block and sex as fixed effects and supplementation period and duration of gestation as covariates.

Results: Maternal supplementation with EFA and EFA+CLA increased the content of ALA in colostrum ($P<0.05$). Concentration of linoleic acid in colostrum was increased by EFA+CLA treatment ($P<0.05$), whereas CLA and EFA+CLA supplementation led to higher concentrations of *trans*-10, *cis*-12 CLA in colostrum ($P<0.05$). First results on fatty acid concentration in blood plasma (13 from 38 calves) showed higher ALA concentration ($P<0.05$) on d 5 in EFA and EFA+CLA than in CTRL and CLA and elevated CLA concentration in CLA and EFA+CLA calves ($P<0.1$). Before first colostrum intake, plasma glucose concentration was higher in EFA than in CTRL and CLA calves ($P<0.05$). An increased insulin concentration was observed 60 min after feeding on d 4 in EFA calves compared to CLA calves ($P<0.05$) and 120 min after feeding in EFA+CLA compared to CTRL calves ($P<0.05$). A reduction of plasma total cholesterol was found 2 h after feeding on d 5 in EFA calves but not in other treatment groups ($P<0.05$). In contrast, HDL-C concentration was decreased in CLA and EFACLA groups by feeding on d 5 ($P<0.05$). Postprandial plasma NEFA concentrations were affected by treatment ($P<0.05$) with lower concentrations in EFA calves than in CTRL and EFA+CLA calves on d 4 ($P<0.1$) and lower concentrations in EFA and CLA calves than in CTRL ($P<0.05$) and EFA+CLA ($P<0.1$) on d 5. Maternal supplementation tended to affect plasma concentration of BHB before feeding ($P<0.1$), leading to highest concentration in CTRL and a lower concentration on d 4 in CLA than in CTRL ($P<0.01$).

Conclusions: First results on the metabolic changes in calves with maternal EFA and CLA supplementation before and immediately after birth indicated variable effects on the glucose and lipid metabolism with a trend for an improved energy metabolism in neonatal calves when dams were supplemented either with EFA or CLA.

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Are rumen-protected *n*-3 and *n*-6 fatty acids differently incorporated into different bovine adipose tissues?

Werden pansengeschützte n-3- und n-6-Fettsäuren unterschiedlich in verschiedene bovine Fettgewebe eingelagert?

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Due to their important and different physiological functions, *n*-3 and *n*-6 fatty acids (FA) have been intensively studied for benefits in animal health and animal-source foods. In order to facilitate the incorporation of dietary unsaturated FA into ruminant body tissues, rumen protection techniques have been developed to minimise their biohydrogenation (saturation of unsaturated FA) in the rumen. Data on the transfer efficiency of these FA comparing the same type of tissue are scarce due to time and effort. Recently, Wolf et al. (1) demonstrated a significantly different incorporation of *n*-3 FA, but not of *n*-6 FA, in three muscles of different function. Therefore, in the present study the partitioning of long-chain *n*-3 FA was investigated also in different adipose tissues from ruminants in comparison to their metabolic competitors, the *n*-6 FA.

Methods: Eighteen Angus heifers each received 7 kg dry matter/day of a basal 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 with 0.45 kg/day of a novel type of coated lipids (Erbo Spraytec AG, Bützberg, Switzerland). Nine heifers received coated fish oil rich in *n*-3 FA (FO diet), the remaining nine heifers were offered coated sunflower oil rich in *n*-6 FA (SO diet). Tissues selected were the intermuscular fat (IMF), the subcutaneous fat (SCF), the cardiac fat (CAF) and the perirenal fat (PF). The fatty acid profile of each of these tissues was analysed in detail by gas chromatography. Data were analysed in R (R Core Team, 2016) using the linear mixed model (package lme4) considering diet type, type of adipose tissue and their interaction as fixed effects, and animal and slaughter date as random effects.

Results: The CAF contained higher proportions (g/100 g of total FA) of *n*-3 and *n*-6 FA than IMF, SCF and PF (*n*-3: 1.4, 1.1, 1.0, and 1.0, respectively; *Pn*-6: 1.2, 1.0, 0.9, and 0.9, respectively; *Pn*-3 FA was overall increased by exchanging SO for FO (0.8 vs. 1.4; *Pn-6*/*n-3* FA ratio decreased (1.2 vs. 0.7; *PPn*-6 FA. The PF and the CAF contained higher proportions of saturated FA than IMF and SCF (65, 63, 56, and 48, respectively; *PP*

Conclusion: Similar to different muscles (1), there was a tissue-dependent diet effect for the incorporation of *n*-3 FA, but not for *n*-6 FA. This might be related to the need for specific FA for a distinct metabolism in CAF whereas for PF no specific function of the *n*-3 FA seems apparent. The different degrees of saturation of the adipose tissues may point towards different physiological functions of CAF and PF compared to IMF and SCF, where especially the last also has the function of body temperature insulation. The very low *n*-6/*n*-3 FA ratios found in all adipose tissues indicate that beef products rich in fat from animals fed a rumen-protected *n*-3 or *n*-6 FA diet, is to be considered more favorable for human health.

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Increasing sainfoin proportion in dairy cows diets modifies fatty acid profile in milk

Erhöhung des Esparsettenanteils in Milchkuhrationen verändert das Fettsäurenmuster in der Milch

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Since several years, use of forage legumes such as sainfoin is gaining interest. Besides its good nutritional values, sainfoin contains condensed tannins which can have a positive impact on ruminal fermentations and milk quality, especially milk fatty acid composition. A major part of the dietary poly-unsaturated fatty acids (PUFA), especially linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are biohydrogenated to saturated fatty acids (SFA) by microbial enzymes in the rumen. Previous studies with 16% sainfoin in the diet suggested a reduction in ruminal biohydrogenation leading to greater 18:3n-3 content in milk and cheese (1; 2). However, it remains unclear whether the reduction in biohydrogenation persists overtime and is dependent on the dietary inclusion level of sainfoin. The objective of the present study was to follow milk fatty acids profile of cows fed different proportions of sainfoin in the diet over 6 weeks.

Methods: In a feeding experiment with 18 Holstein cows (milk yield: 21.6 ± 4.3 kg/d; 230 ± 64 d in milk) were allocated in 3 treatment groups: 0% sainfoin (S0), 20% sainfoin (S20) or 40% sainfoin (S40). For 6 weeks, each group had *ad libitum* access to a total mixed ration (TMR) consisting of grass hay, sainfoin hay, grass silage, sainfoin silage and concentrate at different proportions as follows: S0: 54:0:37:0:9; S20: 49:11:21:10:9 and S40: 43:22:7:19:9.Feed intake and milk production were monitored daily. For each cow, one milk sample was collected at the beginning of the experiment which served as a reference. Subsequently, samples were taken weekly for 6 weeks. The chemical composition of the feed was analysed according to standard protocols. The fatty acid profile was determined by dissolving the milk fat in hexane, followed by transesterification and a separation of the fatty acids methyl esters by gas chromatography. Data for feed intake, milk yield and n-3 transfer rate were treated as repeated measurements and analysed with the MIXED Procedure of SAS considering group, week and the group × week interaction as fixed effects. Statistical analysis for fatty acid profile was based on the differences between samples collected for 6 weeks and the reference sample for each cow, using the MIXED Procedure of SAS with group, week and the group × week interaction as fixed effects.

Results: Dry matter, protein, NDF, ADF, SFA and mono-unsaturated fatty acid (MUFA) intakes were not affected ($P > 0.05$) by groups. Nevertheless, 18:3n-3 intake and consequently PUFA intake was lower ($P \leq 0.05$) in S40 than S0, with intermediate values for S20. Milk yield and ECM were not affected ($P > 0.05$) by treatment groups unlike milk fatty acid profile which was affected ($P < 0.05$) by inclusion of sainfoin in the TMR. Proportion of total CLA and in particular 18:2c9t11 increased ($P < 0.05$) in S20 and S40 compared with S0. In addition, inclusion of sainfoin increased ($P < 0.05$) 18:1t11, 18:3n-3, total n-3 FA and PUFA proportion in a dose-dependent manner with greater values for S40. Milk from cows fed S40 contained lower proportion of SFA due to lower ($P < 0.05$) levels of 16:0 and greater ($P < 0.05$) levels of 18:0, 18:1c9, 18:2n-6, total n-6 and MUFA compared with S0 and S20. Experimental week had only an effect ($P < 0.05$) on some fatty acids, such as 20:4n-6 and 20:5n-3. Finally, n-3 transfer rate linearly raised ($P < 0.05$) with increased dietary sainfoin level (0.038; 0.048 and 0.060 for S0, S20 and S40, respectively). This transfer rate was lower ($P < 0.05$) in the first week compared with the other weeks. In addition, there was a group × week interaction ($P < 0.001$) for n-3 transfer rate.

Conclusion: Increasing proportions of sainfoin in the diet positively affected levels of 18:3n-3 and PUFA in milk without any negative effects on milk yield and feed intake. This positive effect of sainfoin persisted over the 6 weeks of experiment.

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W1

Dietary fibre analyses in a nutritional and physiological context – past and present

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Dietary fibre represents the fraction of carbohydrates along with the non-carbohydrate polyphenolic ether lignin that is not digested by endogenous enzymes in the small intestine of non-ruminant species. Some of the chemical structures in the dietary fibre fraction have a very complex composition, and traditionally, fibre has been considered a marker for low digestibility and nutrient utilisation of feed for animals [1, 2]. Dietary fibre also possesses a number of functional properties in the gastrointestinal tract: 1) impact of soluble fibre on digestion and absorption of nutrients in the small intestine, 2) influence of fibre on rate, extent and type of products formed during fermentation in the large intestine, and 3) influence of fibre on passage rate in small and large intestine. All in all, dietary fibre influences digestion, absorption and metabolism at all sites of the gastrointestinal tract, thereby potentially influencing gastrointestinal health, whole body metabolism and animal health and welfare [3, 4].

The main purpose of the paper is to give an overview of dietary fibre analyses in a nutritional and physiological context. The paper is not a thorough review but a collection of information with citations to other literature where original literature can be found.

Definitions and terminology

Following more than a quarter of a century of debate, a working group established by Codex Alimentarius agreed on a physiological definition of dietary fibre in human nutrition as: "*carbohydrate polymers with ten and more monomeric units which are neither digested nor absorbed in the human small intestine"* [5, 6]. The Codex Alimentarius definition was later on essentially adopted by the European Commission but was extended to include non-digestible oligosaccharides with a degree of polymerization 3-9 and the non-carbohydrate polyphenolic ether lignin [6, 7]. The new extended definition includes not only what has classically been considered dietary fibre, but all carbohydrates not digested by endogenous enzymes in the small intestine, i.e. non-starch polysaccharides, resistant starch, non-digestible oligosaccharides and the non-carbohydrate polyphenolic ether lignin. The difference between the definition of dietary fibre adopted by the European Commission and the Codex Alimentarious is essentially the question of the inclusion of lignin (European Commission) or not (Codex), and whether or not to include non-digestible oligosaccharides with a degree of polymerization of 3-9 [6].

In animal nutrition, we have not yet had a proper discussion on whether or not to adopt the Codex and European Commission definitions on dietary fibre but with the many similarities in terms of gastrointestinal physiology and metabolism between humans and non-ruminant species, it is of relevance to use the recent definition of dietary fibre in animal nutrition, particularly for non-ruminant species. However, most of the published literature on dietary fibre in feed is at present reported as either non-starch polysaccharides, non-starch polysaccharides plus lignin, total fibre, neutral detergent fibre, acid detergent fibre or crude fibre [8]. In the following, the term **dietary fibre** will be used when referring to the extended definition and **fibre** when referring to results generated by the other methods.

Classification and terminology of carbohydrates based on physiology

A principal challenge when classifying dietary carbohydrates by their chemical composition in relation to physiology is relating the various chemical divisions according to the site of digestion [9]. Another classification of the carbohydrate fraction is, therefore, based on a division of the carbohydrates into carbohydrates that can potentially be digested by endogenous enzymes (digestible carbohydrates) and carbohydrates that can potentially be fermented by the microflora (non-digestible carbohydrates) [9]. Digestible carbohydrates include sugars and starch, whereas non-digestible carbohydrates include non-starch polysaccharides, resistant starch and non-digestible oligosaccharides. Non-digestible carbohydrates plus lignin are named dietary fibres as defined by the European Commission [7].

Dietary fibre and physicochemical properties of feedstuffs

The main non-starch polysaccharides are cellulose, and a variety of soluble and insoluble non-cellulosic polysaccharides: β-glucan, arabinoxylan, xylans, xyloglucans and pectic substances to mention the major ones [8, 10]. The dietary fibre content of feedstuffs varies widely with cereals generally having a lower concentration than of legumes and protein rich crops and with a general higher concentration of dietary fibre in co-products from cereals and products from the agro and food industries (Figure 1) [11-13]. There is also

a wide variation in the composition of the non-cellulosic polysaccharides among the feedstuffs; in cereals arabinoxylan and β-glucan are high and β-glucan responsible for the relative high level of soluble non-cellulosic polysaccharides in barley and oats as compared to wheat and corn [8, 10]. In legumes, protein crops and fibre rich materials, pectins and xyloglucans are the main non-cellulosic polysaccharides and the pectin responsible for the relative high levels of soluble non-cellulosic polysaccharides in these feedstuffs [8, 10]. The concentration of resistant starch is higher in legumes (peas and fava beans) than in cereals [8]. Legumes, in general, have also the highest concentration of non-digestible oligosaccharides among the feedstuffs [8]. So, by combining feedstuffs there are many possibilities for obtaining diets with different dietary fibre composition.
The fibre values measured on the same feedstuffs varies depending on the methods of analysis [8]. For all classes of feedstuffs, dietary fibre gives the highest values because it includes all dietary fibre components including non-digestible oligosaccharides, fructans and resistant starch.

Neutral detergent fibre values are consistently lower than the total fibre values but relatively close to the insoluble fibre components (sum of lignin, cellulose and insoluble non-cellulosic polysaccharides). The acid detergent fibre values are close to the values for cellulose and lignin, whereas the values for crude fibre are consistently the lowest for all groups of feedstuffs [8].

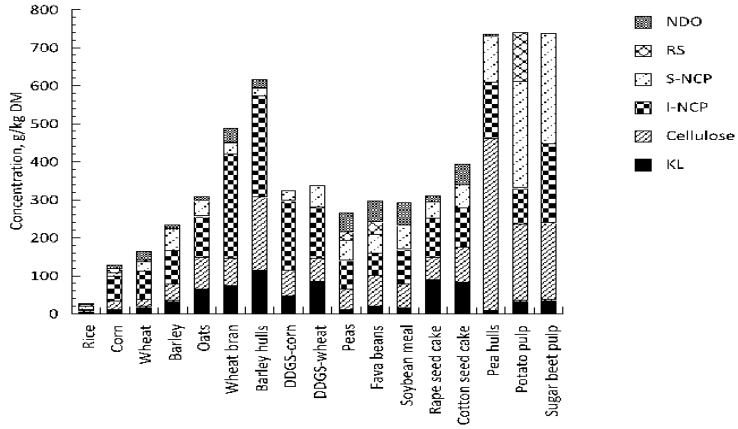

Figure 1. Dietary fibre concentration (g/kg DM) in different feedstuffs. NDO, non-digestible oligosaccharides; RS, resistant starch; S-NCP, soluble non-cellulosic polysaccharides; I-NCP, insoluble non-cellulosic polysaccharides; KL, Klason lignin.

A common feature of all dietary fibre sources is the ability to swell, hold water in the cell wall matrix and to increase viscosity when exposed water. These aspects were recently studied using an In vitro methodology simulating the digestion conditions in stomach and foreguts (stomach and small intestine) [14]. Barley and wheat in a basal diet were substituted by increasing levels of barley hulls, pectin residue, sugar beet pulp and potato pulp, respectively, at four levels of fibre from 225 g/kg DM to 450 g/kg DM. The readout was water binding capacity and swelling in stomach and foreguts. It was found that the fibre sources with the highest content of soluble fibre (sugar beet pulp and potato pulp) resulted in substantially higher increases in water binding capacity and swelling than barley hulls high in insoluble fibre [14]. However, while all dietary fibre sources swell and hold water, the viscous properties depend on the type and chemical nature of the polysaccharides making up the dietary fibre fraction. For instance, sugar beet pulp will primarily increase the water binding capacity of digesta whereas the viscosity elevating properties of sugar beet pulp are low [15]. Contrary to that, β-glucan and arabinoxylan located in the endosperm cell walls of cereals will to a larger extent solubilise from the cell wall matrix and raise luminal viscosity [15].

Influence on digestion and fermentation processes

The gastrointestinal tract consists of different compartments – mouth, stomach, small intestine and large intestine – and supplying organs – liver, pancreas – involved in the digestion and absorption processes. The

whole assembly is integrated with the peripheral organs through a large set of receptors (distension, tactile, chemo) that monitor the digestive and absorptive processes through neural and hormonal feedback signals. In this way, variations in blood nutrient content are minimised, and the provision of nutrients to the different organs are regulated and optimised.

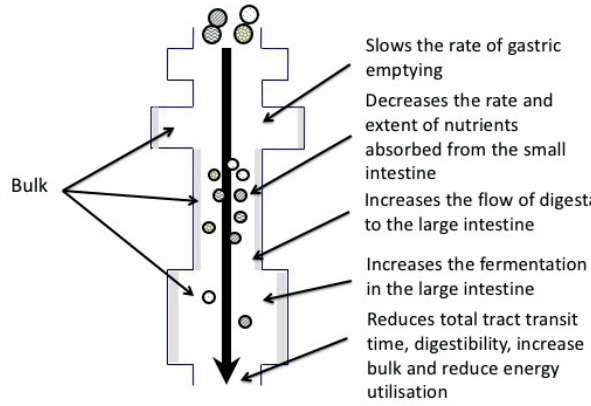

Figure 2. Influence of dietary fibre on the physiological processes related to transit time, digestion and absorption processes in the various segments of the gastrointestinal tract and bulk and energy utilisation.

Dietary fibre influences the digestion and absorption processes at all sites of the gastrointestinal tract as well as the secretion of fluids from pancreas and liver (Figure 2). Raised dietary fibre increases the weight and content of the gastrointestinal tract and leads to a higher flow of nutrients at all sites [3]. Soluble dietary fibre will raise luminal viscosity, thereby prolonging gastric emptying and interfering with the digestion processes in the small intestine by hindering the contact between the substrate and digestive enzymes and by slowing down the movements of hydrolytic products of the digestion processes. Soluble as well as insoluble dietary fibre will provide substrate for fermentation by the microbiota in the large intestine, but there is a significant difference in how soluble and insoluble dietary fibre is handled in the large intestine. Soluble dietary fibre is readily fermentable; the major part is broken down in caecum and proximal colon while insoluble dietary fibre is degraded at more distal locations, and parts of it, particularly dietary fibre in lignified tissues, will not be broken down at all [3]. The main metabolites formed in consequence of microbial fermentation are short-chain fatty acids (predominantly acetate, propionate and butyrate) which in consequence of their production lower pH of the luminal content [16]. The major part of the produced short-chain fatty acids, however, is rapidly absorbed and provides energy to the host although the utilisation of the energy provided as short-chain fatty acids is lower than that of the energy absorbed as e.g. glucose from the small intestine. The reason for the lower energy utilisation of short-chain fatty acids compared to glucose can be found in loss of fermentation gasses and a lower utilisation of short-chain fatty acids in the intermediate metabolism. Therefore, high dietary fibre diets have in general a lower energy content than low dietary fibre diets which is primarily due to a lower digestibility but is also due to a lower utilisation of absorbed energy [3].

Influence of dietary fibre on intestinal and metabolic health

Several of the abovementioned effects of dietary fibre related to digestion and fermentation processes may influence intestinal and metabolic health [4, 16]. A thorough discussion of those effects is outside the scope of the current short paper, but among the effects of dietary fibre can be mentioned: prebiotic effects, i.e. selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improving intestinal and host health, stimulation of total and specific short-chain fatty acids where particularly the stimulation of butyrate may have positive effects not only on the epithelial cells lining the large intestine but also on metabolic health parameters [17, 18].

Conclusions and perspectives

By the advance of the analytical techniques for the characterisation of carbohydrates including dietary fibre it is possible to obtain a more detailed knowledge on the different dietary fibre components in the feeds and how they potentially can influence digestion and absorption and the fermentation processes in the gastrointestinal tract all of which are important aspects in relation to understanding the effects of the feed on intestinal and metabolic health.

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W 2

Modulatory abilities of dietary fiber on gut function – beneficial or physiologically costly?

Zur modulierenden Wirkung von Nahrungsfaser auf die Darmfunktion – physiologisch wertvoll?–

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Dietary fiber (DF) including resistant starch has a contradictory status in monogastric nutrition and is often characterized as an anti-nutrient due to their negative effects on nutrient digestibility and feed conversion efficiency (1). In human nutrition, in turn, DF gains popularity due to its effects on intestinal health and is increasingly regarded as functional food (1). When comparing the effects of DF on gut function in monogastric animals, it is essential to differentiate the physiological effects between soluble and insoluble DF sources. Soluble and insoluble DF differ in their rheological properties and fermentability, which are as important for the gut physiological response as the chemical structure. For instance, from low to high viscosity DF affects the kinetics of digestion and absorption, and from slowly to rapidly fermentable DF alters the production of short-chain fatty acids (SCFA) (1). These properties also affect the digestion and absorption of other nutrients and the gut microbe-host-interactions. Changes in nutrient flow after DF consumption affect whole body nutrient use, such as protein and lipid deposition, nutrient excretion and health and alter the nutrients available for microbial fermentation (1). In this respect, many effects of DF are mediated via their modulatory abilitity on the gut microbiota and generation of microbial metabolites, which are hard to distinguish from direct effect of the DF on the gut mucosa. Matrix effects and interactions with other dietary components can further modulate the intestinal host-related and microbial effects. Moreover, conclusions drawn in studies using purified DF sources may not be transferable to natural feedstuffs.

Increasing the amount of DF in the diet for pigs and poultry commonly reduces the nutrient digestibility in the small intestine and leads to greater endogenous nitrogen losses, depending on DF level, type and water-holding properties (1). In general, soluble DF impairs protein digestion and absorption more than insoluble DF in pigs and chickens (2, 3). The decrease in nutrient digestibility by soluble DF is due to an increase in digesta viscosity that limits the access of endogenous enzymes to nutrients and affects digesta passage rate (4). Insoluble DF impairs digestion by enhancing digestive secretions and decreasing the retention time of the solid and liquid phase in the small and large intestine of growing pigs (5). Negative effects caused by a high-viscous DF on digestion may be abrogated if the DF is also highly fermentable. To give an example, high-fermentable high-viscous soluble DF, such as oat β-glucan, did not negatively impact ileal digesta viscosity and digesta passage to the same extent as a low-fermentable high-viscous DF source, i.e. carboxymethylcellulose, in pigs, because oat β-glucan was already largely fermented in pig's stomach (6). As in the case of carboxymethylcellulose, a longer retention time caused by increased digesta viscosity may have beneficial effects, by increasing apparent ileal nutrient digestibility in pigs (6). However, carboxymethylcellulose also enhanced the intestinal proliferation of opportunistic pathogens, such as enterotoxigenic *Escherichia coli*; therefore, its negative effect on gut health annihilated the positive effect on nutrient digestibility (7). Chickens are especially susceptible to high-viscous DF, such as pectin, whereas cellulose, as an example for insoluble DF, seems to be more or less inert in regard to intestinal nutrient availability (3). Findings for effects of soluble and insoluble DF on the intestinal structure and mucus production are contradictory in the literature. Some studies found no effect on the intestinal morphology or goblet cell quantity and type, e.g. in chickens (3). Others, in turn, reported that high digesta viscosity caused by soluble DF stimulated epithelial cell proliferation, whereas insoluble DF increased mucus secretion in the small intestine due to mechanical effects on the gut wall, damaging the mucus layer (1, 8).

Certain DF types, such as β-glucans and resistant starch, can act as prebiotics, beneficially affecting the host by selectively stimulating the growth of specific bacteria (i.e. *Lactobacillus*, *Ruminococcus* and *Faecalibacterium*) as well as fermentation in the large intestine (9). In particular the resistant starch fraction has been reported to induce positive effects on pigs through SCFA production, mainly butyrate and propionate, with profound effects on gut health as energy sources, inflammation modulators, vasodilators and on gut motility (10). SCFA influence intestinal gene expression through inhibition of histone deacetylase and by binding to fatty acid-sensing G-protein-coupled receptors (11). These receptors play crucial roles in the promotion of gut homeostasis and modulate intestinal secretion of hormones, such as peptide YY, gastric inhibitory polypeptide, and glucagon-like peptide 1 (10), which have been linked to increased satiety in pigs and humans.

Restrictively fed pigs, such as gestating sows or finishing pigs, may suffer from hunger and related welfare problems and may therefore benefit from satiety enhancing abilities of DF (12). For instance, pectin was the least satiating fiber compared to lignocellulose and resistant starch when included in the diet of adult sows (12). Moreover, SCFA produced during fermentation of DF can attenuate inflammatory processes via activation of G-protein receptors by down-regulating the expression of pro-inflammatory mediators, such as pro-inflammatory tumor necrosis factor-α (TNF-α) and nuclear transcription factor kappa-light-chain-enhancer of activated B cells (NF-KB) (11). In addition, SCFA, especially butyrate, stimulate intestinal mucus secretion, thereby protecting the epithelium from abrasive damage and enhancing the non-specific mucosal barrier (8).

In conclusion, due to the ambivalent nature of the effects of DF on gut function, depending on the DF type, source and inclusion level, it will be important for future diet formulation to find a proper balance between the exploitation of the prebiotic capabilities of DF and related benefits for gut function and metabolism and the need to feed pigs and poultry for efficient production.

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W3

Dietary fiber – its diverse effects on pig's health

„Nahrungsfaser" – ihre vielfältigen Effekte auf die Gesundheit von Schweinen

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Introduction:

The term `dietary fiber´ is not a common one in animal nutrition, but is used in nutrition of human beings with diverse definitions. In contributions presented at this workshop before, a deeper characterization of this term was given (Bach Knudsen). Thus in the following the `dietary fiber´ summarizes carbohydrates degraded by intestinal microorganisms only – and not by host own enzymes (Bach Knudsen et al. 2016). It has to be underlined that also `resistant starch´ is included on this way and often dietary fiber exceeds markedly the amounts of `crude fiber´ defined by the procedure in the Weender analysis (compare oats and rye!). Südekum (2009) emphasized that the traditional analyses of crude fiber, N-free extracts is not sufficient in future times and that an adequate differentiation of the carbohydrate fractions is required for a deeper understanding of their diverse effects within and beyond the gastrointestinal tract (GIT).

Even more complicated is the term `pig's health´: It is used to describe the status of individuals but also of herds. `Health´ is more than the absence of diseases. The term is not used only, when there are disturbances, alterations, dysfunctions of organs but is more and more used in the sense of `welfare´ and `wellbeing´; in human medicine there is the term `mental´ health, but up to now in veterinary medicine terms like behavioural disorders are preferred. But without any doubt there are problems in animal production, i.e. in food producing as well as in companion animals, that are related to abnormal behavior (Kamphues a. Rieger 2017). Whenever the role of feeds and feeding is on debate the focus lies on the health of the GIT. The term `gut health´ is nowadays more frequently used. Therefore it is mandatory to characterize it deeper: A healthy GIT combines a normal development (including size, morphology and histology), physiological functions (digestion / absorption / excretion), an undisturbed microflora (species diversity, counts of bacteria, activity, eubiosis, without pathogens) and `normal´ reactions (including defense / immunology) on / against strange substances and organisms. In pigs, diseases and conditions such as swine dysentery, post-weaning diarrhea, gastric ulcers and salmonellosis are known to be affected by the diet and dietary fiber can impact on the incidence of post weaning diarrhea and swine dysentery markedly (Pluske 2016).

`Dietary fiber´: diversity of effects

Regarding the diversity of effects there is a need of differentiation: At first there are effects on the feed / diet itself (`bulky diets´; Loisel et al. 2013), secondly on the fate of the diet throughout the intestinal tract and finally systemic effects beyond the GIT due to constituents and / or metabolites.

- effects on the diet itself

Depending on the chemical composition of dietary fiber (soluble / insoluble in water) there are consequences for the physicochemical properties, structure and texture (`physically effective fiber´; AfBN 2014) as it is known for rations used in dairy feeding. Also there are marked influences on feed technology (for example feeds' properties regarding grinding, compaction). There are differences between grains regarding the particles' size after grinding although identical techniques are used (hammer mill, 3mm holes). GRONE et al. (2018) observed differences especially between barley and wheat, the share (%) of particles < 0.2 mm amounted 10.2 ±2.83 and 18.0 ±1.08 respectively. Thus, it is not astonishing that in general there is a trend for higher prevalence of gastric ulcer in pigs when wheat levels in diets for fattening pigs are increasing (Wolf a. Kamphues 2007). Furthermore, the energy and nutrients` density within the diet are affected, also diets` consistency when the diet is offered in a liquid form for example. The dietary fiber composition and content determine especially the digestibility rate of nutrients of the feed material, of diets and rations, too.

- dietary fiber and the fate of the diet throughout the intestinal tract

Depending on composition and content of dietary fiber there are marked effects on the process of ingestion (speed of intake, need for chewing activity, saliva production). Insoluble lignified fiber of higher coarseness is required to prolong the time for feed intake (for example to avoid behavioural disorders; Kamphues 2015),

to favour the intensity of chewing, and to stimulate saliva production (as known for ruminants since centuries). The physical form of the diet is depending on both the dietary fiber and the intensity of diminution (Betscher et al. 2011). Furthermore the organ size and mass are depending on both factors as known for the development of the rumen in calves, for stomach and pancreas in pigs (Arlinghaus et al. 2012; Cappai et al. 2016) and poultry. Last but not least the passage time of ingesta is related to dietary fiber (soluble carbohydrat → viscosity → transit times) and physical form of the diet (Bach Knudsen et al. 2012). Higher fiber levels in the diet prolong the time of digesta in the stomach and small intestine, but result in a faster passage through the hindgut (Drochner a. Coenen 1986). Both effects have an impact on the time of exposure to host own enzymes but also to ones of microbial origin.

Related to the composition of the dietary fiber there may be further influences like on the viscosity of the digesta in the gastrointestinal tract, localization of digestion (proportion in prececal versus postileal parts (water soluble fiber!), microbial degradation and fermentation patterns, and the excretion (process of defaecation; quality of faeces (Leurs 2016)/ excreta; prevention of diarrhea (Molist et al. 2014 → weanling pigs) and constipation (Fuller et al. 2016; Oliviero et al. 2009; Warzecha 2006). Especially the gut fill is depending on dietary fiber level due to the indigestible parts of the diet, but also because of the water holding capacity of dietary fiber. Especially in feeding pregnant sows with concentrates only there is a need to meet the official minimum requirements regarding `crude fiber´ levels in the diet (≥ 8 % of dry matter; Kamphues 2015). At this higher fiber levels the diets have a more bulky character, it means that higher amounts of concentrates can be offered resulting in higher gut fill and less risks for stereotypies and further abnormal behavior (Robert et al. 1993). There are several experimental studies in sows on the advantages of using sugar beet pulp in compound feeds (Schade 2000). In comparison to further fiber rich feed materials there is a quite high digestibility of organic matter (~ 80 %; DLG e.V. 2014), and a high postileal fermentation resulting in increased proportion of acetic acid. According to Souza da Silva et al. (2012) lignocellulose and resistant starch seem to be the most effective fiber materials for the `feeling of satiety´ in pigs. Finally there are marked effects of dietary fiber on the composition and activity of the gastrointestinal flora (Leurs 2016).

- dietary fiber and fermentation pattern in the GIT

For decades it is well known that the fermentation pattern due to microbial degradation in the alimentary tract depends on the chemical composition of dietary fiber. For example this knowledge has been used in feeding dairy cows to favour the *acetate* production via increasing cellulose and hemicellulose intake or to increase the *propionate* production via higher starch contents in rations for fattening bulls.

In the last ten years more and more interest has been given to the third important volatile fatty acid, i.e. to *butyrate* by dietary means: substances like polyfructans and arabinoxylans are suitable to increase the formation of butyrate by the intestinal microflora (Bach Knudsen et al. 2012; Bedford a. Gong 2017; Kamphues et al. 2017). What are the main reasons for the increased interest in a higher butyrate intake (via additives) or butyrate production within the GIT? At first there is the trophic function which butyrate has for the epithelia of the GIT mucosa. But there are lots of further effects due to higher butyrate levels in the digesta: These are ranging from the control of important processes such as proliferation, differentiation and apoptosis (retarded!) of the epithelia, the control of cytokine production (process of inflammation), a stimulation of certain immune cells (including their control), up to `signal effects´ on certain bacteria (including pathogens) in the intestinal tract (lowering the translocation of Salmonella from the digesta into the animal; Guilloteau et al. 2010). Whenever it is intended to achieve a higher ileocaecal flux of organic matter / nutrients / starch a lower grinding intensity of cereals is recommended. Favouring the influx of starch into the hindgut means that within the fermentation process the formation of propionic acid but also of butyric acid will be favoured. That is for example the main reason for using diets of a lower grinding intensity when a higher prevalence of Salmonella is the specific challenge on farms (Visscher et al. 2009). Finally there are hints on systemic effects when higher levels of butyrate in the blood / serum (Bach Knudsen et al. 2005) are achieved, that might be related to the feeling of `satiety´ or of `sadness´. In recent literature interesting effects regarding mucus formation, favouring the intestinal barrier (tight junction proteins ↑) and production of host defense peptides due to higher butyrate levels were described (cited by Kamphues et al. 2017). According to Stilling et al. (2016) butyrate plays a central role in the exchange of information between processes in the GIT and the brain. Especially in depressive like behaviour there are chances for modifying and treatment via butyrate

application. There are also former experimental studies in pigs indicating that `resistant starch´ (belonging to dietary fiber) might be more effective than `crude fiber´, resulting in a higher gut fill (Serena et al. 2009). There is the interesting question, whether the behavior / feeling of satiety is more related to the gut fill (caused by `crude fiber´) or more to the higher formation of butyrate (for example due to `resistant starch´; Sapkota et al. 2016). The answer might have a massive impact on diet formulation, for example especially for pregnant sows and further animals fed restrictively.

Perspectives for distinct compounds (sources) of dietary fiber

Depending on the diverse substances belonging to `dietary fiber´ there are chances to use them specifically, it means depending on the intended goal (`selective inclusion of dietary fiber´, Jha a. Berrocoso 2015; `prebiotic effects´, Lindberg 2014). Thus, whenever it is intended to have a `diluted´ diet – for example in feeding pregnant sows with higher amounts of feed - it is recommendable to use ingredients with higher contents of lignified crude fiber. The same is valid, when it is intended to have a prolonged time of feed ingestion; on the other side it could be an advantage to use non-lignified fiber (but not pectins), when a higher butyrate formation in the hindgut ingredients like topinambur, chicoree or rye are recommendable. Of interest for avoiding `boar taint´ by reduced formation of skatol in the hindgut there could be a chance for an `old´ type of grain, i.e. for rye, because of its high polyfructan and arabinoxylan content favouring specifically the butyrate production in the hindgut (Kamphues et al. 2017).

Summary/conclusion

The important role of dietary fiber and its physicochemical properties for maintaining rumen health and well-being is recognized for centuries. Regarding monogastric species it seems there is a change in the evaluation and judgement. In former times the `diluting´ effects of dietary fiber were underlined whereas nowadays the benefits of dietary fiber for the health of the GIT are more and more emphasized. Especially the butyrate production by the intestinal microflora is of interest regarding gut health and -maybe- related to behavioural disorders, a real challenge in modern production systems of swine. Furthermore it is questionable whether dietary fiber could display effects that are related to non-digestible parts of the diet and their primary effects. Finally it should be emphasized that there are interesting findings regarding a delay of dietary fiber effects, for example on colostrum quality (after feeding high fiber diets during pregnancy) or on piglets behaviour before rearing (Loisel et al. 2013; Bernardino et al. 2016).

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W4

The role of fibre with respect to feeding behaviour

Nahrungsfaser im Kontext mit dem Fressverhalten

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Normal behaviour and behavioural needs

Feeding behaviour, as all species-specific behaviour is the result of millions of years of evolution. Although domestication has changed the animals as a selection towards human desires has taken place, during this process, no completely new behaviours appeared by domestication and no behaviours completely disappeared [1]. Therefore, todays domestic pigs show all behavioural patterns of wild pigs. This holds also for pigs which grew up in common conventional housing conditions when brought into a semi-natural environment [2]. This knowledge is of special importance when the behavioural needs and normal behaviour of the animals have to be defined. Normal behaviour is defined as the quantity and quality of the behaviour of the wild form or else the behaviour of the domesticated form living in a semi-natural environment [3]. Behavioural needs arise by those behaviours that were of special importance in the evolution process of that species.

Over time, pigs have adapted to living in forest areas. They live in stable matriarchal groups, whereas the grown-up males live solitary and join the group for breeding season. Within the group, there is a stable rank order. The behaviour is highly synchronized. Thereby, the group follows a diurnal rhythm, which is also dependent on exogenous influences.

As pigs are omnivorous animals, they spend most of their time foraging for food. The food they ingest is of medium energy content, whereby food with high energy is preferred, but rarer. Mainly, plant food such as grass, roots, seeds, leaves and fruits as well as mushrooms are ingested, but also insects, carrion or small live invertebrates and vertebrates are ingested, if the possibility arises. Pigs are dependent on the continuous exploitation of food sources which leads to a high motivation to explore the surroundings. The foraging behaviour consists of exploration with the snout which is about as good innerved as human fingertips. Furthermore, rooting behaviour as well as pawing with the front legs are part of the foraging behaviour. For the rooting behaviour, the anatomy of the snout is perfectly adapted [4].

Wild pigs spend around 40 % of their day with foraging and feeding behaviour [5] and 50 % within 24 h with resting. In conventionally stabled fattening pigs, the amount of foraging and feeding behaviour is significantly lower (1-10 %). Here, the animals spend more time resting (80 %) [3]. About the same time amounts were found for group housed pregnant sows [6]. Lactating sows spend even more time of the day resting (85 %) and only 5 % with foraging and feeding behaviour. However, pregnant and lactating sows in outdoor husbandry systems on pastures without nose rings or clips spend again around 30-40 % of their day with foraging behaviour and feed intake. Also, if conventionally reared sows are kept in semi-natural environment, they change their behavioural patterns from around 80 % resting to only 50 % resting at night time and noon and around 40 % foraging and feed intake. This also holds when the animals are additionally fed ad libitum [2]. Thus, foraging behaviour is highly motivated and the desire to explore extremely high. In summary, important behavioural needs for pigs are the need for social contacts and the need to explore.

Consideration of behavioural needs in husbandry

From the great social needs and synchronization of behaviour within a group, in combination with the high desire for foraging and feed intake, a high jealousy about food can arise. Most of the agonistic events in husbandry can be observed near the feeding through [7]. It is therefore most species appropriate if all pigs in the group can feed simultaneously [8]. On feeding stations with a feed space : animal ratio of 4 : 1, the time spent at the through varies from 1-9 % of the time within 24 h, which is dependent on the rank order. Furthermore, the access to the through can be blocked by higher ranked animals [9]. As the natural diet is versatile, from an ethological point of view, this should also be aimed for in husbandry. The high motivation to explore is accounted for in husbandry by the provision of material for occupation. It is important to consider the behavioural needs thereby: First of all, the material must be modifiable. Moreover, it is best to provide the material hanging to avoid soiling. The longer the material stays in the pen, the less attractive it is for the pigs, i.e. the provision of changing and constantly new material is beneficial [10]. It is further proven that organic material is preferred above inorganic material [11], which can also be due to the higher deformability potential of organic material. The less adequate the enrichment material is, the more time is spent with investigation of pen features such as the floor or other pen mates.

Although in husbandry more social behaviour seems to occur, this is discussed interchangeably by different authors as it is often interpreted as redirected exploration behaviour or compensation for the insufficient fulfilment of behavioural needs [11].

Behavioural needs and behavioural disorders

If the behavioural needs are neglected in husbandry, aggression within the group can occur which results in injuries and diseases. It can further lead to suboptimal feed intake and conversion and therewith to suboptimal weight gains. Furthermore, behavioural disorders can arise. In general, behavioural disorders are an expression of an overstraining of the adaption potential of the animals. This means that especially their behavioural needs are not fulfilled. Despite being discussed controversially in literature, all authors in general agree that behavioural disorders are an expression of some amount of suffering [12, 13].

In the development of behavioural disorders, also the impossibility to follow motivational states plays an important role. Motivation describes a hypothetical internal state that arises when the actual value (e.g. low gut fill) differs from the nominal value (e.g. satiation). Then, appetence behaviour occurs, i.e. the animal searches for the possibility to shift the actual value towards nominal value [14]. If this cannot be achieved despite attempts such as increased exploration behaviour, behavioural disorders may develop [15].

In sows, especially tongue rolling, sham chewing, bar biting and vulva biting are described [13]. Whereas vulva biting occurs especially as result of suboptimal feed provision in feeding stations due to the fact that higher ranked sow bite in the vulva of the feeding animal in the station. The other three described behavioural disorders are seen in the context of insufficient saturation, insufficient provision of fibre and insufficient time spent with feeding [13]. In fattening pigs, the most important behavioural disorders are ear and tail biting. The causes are discussed very controversially. However, experts agree on the fact that there is not one single cause but it is made up of multiple factors causing stress in different ways such as the weaning management, group structure, climate, feed and water provision and quality or the health status [3]. Especially for ear biting, necrotic happenings due to endo- or mycotoxins are described which are also discussed for tail biting [16–18]. Due to this multifactoriality, it is hard to make static suggestions to safely avoid these behavioural disorders. Nevertheless, studies in the last years showed that the best practice to reduce tail biting is the optimisation of management practices. Most importantly, the correct provision of resources such as feed and water should be checked. Secondly, provision of changeable, organic occupation material is proven to have positive effects. Straw is thereby highly preferred by the animals, but also e.g. hay or corn silage can have positive effects. The effects are even better if the material is administered in a daily routine so that it is always new and has not taken on the smell of the stable. Already low amounts of about a handful for 10 animals have these positive effects [19]. Further studies demonstrated that alone the effect of tender and care in the daily rhythm can reduce the occurrence of behavioural disorders as well as stress in novel handling situations [20]. On the other hand, no effects were found by the simple provision of more crude fibre as ingredient in the pelleted feed [21]. This clearly demonstrates that the role of crude fibre in terms of prevention of behavioural disorders should not be neglected: Due to the increased filling of the gastrointestinal tract, a longer lasting feeling of satiation can be achieved [22]. This again leads to calmer animals and can impede the motivation to explore. However, the form of the provision of the crude fibre should go in accordance with the behavioural needs, i.e. the species specific foraging behaviour of the pigs. Thus, the benefits will be increased if it is provided in a form as a new and good smelling occupation material that is chewable, deformable and can be rooted and explored [23] such as the twice daily provision of straw.

Conclusion

In summary, especially the consideration of the behavioural needs which are needs for social contacts and strong motivation to explore is importanr in the context of feeding behaviour. Thereby, one should pay special attention to the time that pigs would usually spend with the feeding behaviour including foraging behaviour. Thus, especially regarding the prevention of behavioural disorders, solutions must be found to provide pigs the possibility to combine again their highly motivated exploration desire with the feed intake. Suggestions such as the “Düsser Wühlturm” for provision of organic enrichment material are feasible solutions. Ways to provide not only the enrichment material in the way to promote rooting behaviour, but also the normal feed intake, are explored but have not achieved practicability up to now as the foraging behaviour is problematic with regard to destruction of pen features. A currently tested example is the “WühlTrog” of “BigDutchman”.

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W5

Dietary fiber displaces no concentrated feed and makes pigs less aggressive

Nahrungsfaser verdrängt kein Ferkelaufzuchtfutter und führt zu weniger aggressiven Schweinen

*Preißinger, W. – Grub, Schwarzenau

Introduction

The composition of feed is not only important for the nutrition supply, but is also a crucial factor for the health of the intestine and for the well-being of the animals. For this purpose, the fiber fraction is essential. Relating to a species appropriate feeding, the Society of Nutrition Physiology points to the importance of fiber in feeding pregnant sows, but a final statement on the required fiber supply level is not yet available for pigs (1). The German Tierschutz-Nutztierhaltungsverordnung requires a daily minimum crude fiber intake of 200 g or 8 % crude fiber (100 % DM) in diets for pregnant sows (2). For the feed of sows, piglets and fattening pigs, the DLG specifies the crude fiber contents given in Table 1 (3, 4). However, a final recommendation on the content of fiber in diets for pigs is missing.

Table 1: Recommendation for crude fiber in diets for pigs (880g DM/kg feed), (3, 4)

	pregnant sows	lactating sows	piglets	fattening pigs
crude fiber (g/kg feed)	≥ 70	50	30-40	>30

The function of fiber in diets for pigs is manifold and depends on the type of the fiber. The function of the fiber has been investigated mainly in diets for pregnant sows. Due to the low nutrient and energy requirements of these animals there is a risk of low total feed intake and associated hunger. This is caused on the one hand by an insufficient filling of the gastrointestinal tract and on the other hand by the temporary change of the glucose or insulin level in the serum (5, 6, 7). The results are stereotypes, aggressions or other behavioral disorders (8, 9). To achieve saturation, fiber or fiber rich diets are recommended (10). But the results in literature are inconsistent. Diets with high levels of crude fiber usually led to a reduction in behavioral disorders, but the effects depend on various factors, such as the type of fiber, the feeding frequency, the age of the animals, feeding conditions and so on (6, 7, 8, 11, 12). Unfortunately, there is a lack of studies on fiber supply in piglets and fattening pigs.

High contents of crude fiber in diets do not reduce performance

At the Bavarian State Research Center for Agriculture numerous experiments were carried out on fiber supply of piglets and fattening pigs. When piglets are fed high levels of crude fiber, this resulted in a numerically better performance and a slightly lower average daily feed intake (Table 2). The feeding of diets with higher contents of crude fiber did not lead to any negative effects (13, 14). In practice, reduced medicinal treatment is reported, when high levels of crude fiber are fed in diets.

Table 2: Feed intake, performance and feed conversion ratio of docked piglets fed with different crude fiber content in the diets (13, 14)

		control group	fiber group
trial 1:	crude fiber (g/kg feed)	32	54
	average daily feed intake (g)	822	809
	average daily gain (g)	494	508
	feed conversion ratio (kg/kg)	1.65	1.58
trial 2:	crude fiber (g/kg feed)	33	47
	average daily feed intake (g)	999	958
	average daily gain (g)	570	577
	feed conversion ratio (kg/kg)	1.74[b]	1.64[a]

Increased fiber content in the diets for fattening pigs achieved by admixing straw, wheat bran or sunflower meal had no effect on performance, feed intake, feed conversion ratio and carcass quality (table 3) (15). Lean percentage was increased when straw was used in liquid feed (16).

Table 3: Feed intake, performance, feed conversion ratio and carcass quality of docked fattening pigs fed with different fiber rich feed in the diets (15, 16)

		control	fiber groups		
		group	straw	wheat bran	sunflower meal
trial 1	crude fiber (g/kg feed)	35	44	36	42
	average daily feed intake (kg)	1.79	1.80	1.76	1.82
	average daily gain (g)	793	769	752	800
	feed conversion ratio (kg/kg)	2.26	2.34	2.36	2.26
	lean (%)	61.4	62.4	61.8	61.7
trial 2	crude fiber (g/kg feed)	32	41	-	-
	average daily feed intake (kg)	2.76	2.75	-	-
	average daily gain (g)	921	897	-	-
	feed conversion ratio (kg/kg)	2.98	3.04	-	-
	lean (%)	60.0[b]	60.8[a]	-	-

trial 1: dry feed; trial 2: liquid feed

Fiber feeding displaces no concentrated feed

When fiber rich feed such as alfalfa hay, grass pellets, straw pellets or corn silage was fed in additional feed troughs, there was also no significant effect on growth or consumption of regular feed (Table 4). However, since consumption of regular feed was numerically increased when fiber rich feed components were fed, for some fiber rich feed this resulted in a significant rise of regular feed conversion ratio (17, 18).

Table 4: Feed intake, performance and feed conversion ratio of docked piglets fed with different fiber rich feed (17)

		Control group	Fiber group
trial 1: alfalfa hay	average daily feed intake (g)[1)]	888	912
	average daily intake of alfalfa hay (g)	-	9 (3-21)
	average daily gain (g)	530	525
	feed conversion ratio (kg/kg)[1)]	1.69[b]	1.75[a]
trial 2: grass pellets	average daily feed intake (g)[1)]	906	927
	average daily intake of grass pellets (g)	-	33 (16-41)
	average daily gain (g)	540	541
	feed conversion ratio (kg/kg)[1)]	1.69	1.72
trial 3: straw pellets	average daily feed intake (g)[1)]	772	799
	average daily intake of straw pellets (g)	-	30 (8-53)
	average daily gain (g)	562	550
	feed conversion ratio (kg/kg)[1)]	1.68[b]	1.78[a]
trial 4: corn silage	average daily feed intake (g)[1)]	936	983
	average daily intake of corn silage (g)	-	10 (7-20)[2)]
	average daily gain (g)	525	516
	feed conversion ratio (kg/kg) [1)]	1.75[b]	1.81[a]

[1)] fiber rich feed not included

[2)] corrected on 800 g DM/g feed

Only small amounts of fiber rich feed were consumed

Figures 1 and 2 shows the intake of fiber rich feed of docked piglets. In both experiments the intake of fiber rich feed was generally low, but it was higher for pelleted supplements compared to hay or silage. At the end of the feeding trial (30 kg body weight), the maximum intake of fiber rich feed accounted for around 50 g per day for pelleted supplements and almost 30 g for silage and hay.

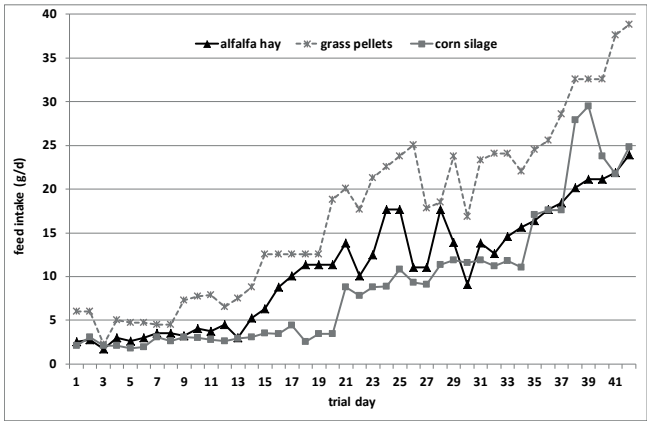

Fig. 1: Intake of alfalfa hay, grass pellets and corn silage (corrected on 800 g DM/kg feed) during the feeding trial (18)

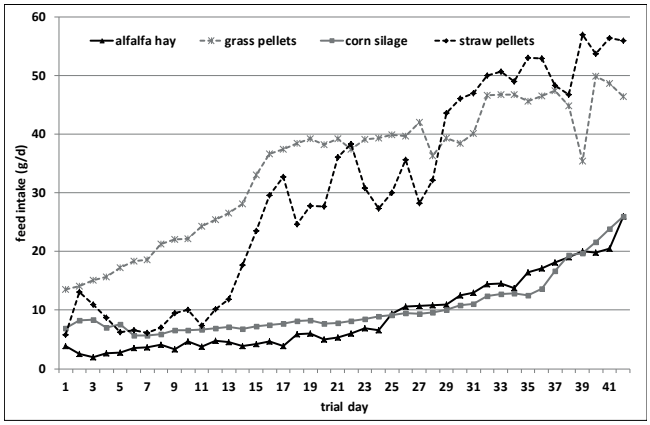

Fig. 2: Intake of alfalfa hay, grass pellets, corn silage and straw pellets (corrected on 800 g DM/kg feed) during the feeding trials (17)

Fiber feeding reduces tail biting

In numerous housing trails with undocked piglets demonstrated that providing eatable organic material is a good measure to reduce tail biting (19, 20). In feeding trails with undocked piglets less tail biting was shown when alfalfa pellets were offered. The mixing of alfalfa pellets into the diets had no effect on tail biting (21). Further experiments with other fiber rich feeds such as sugar beet pulp or straw pellets are necessary.

Table 5: Feed intake, performance, feed conversion ratio and tail biting of undocked piglets fed different fiber rich feed in extra troughs (21)

	Trial 1		Trial 2	
	alfalfa pellets, extra	control	alfalfa pellets, extra	alfalfa pellets, mixed in diet
average daily feed intake (g)[1)]	1109[a]	947[b]	925	870
average daily intake alfalfa pellets (g)	80	--	60	--
average daily gain (g)	575[a]	543[b]	486[a]	453[b]
feed conversion ratio (kg/kg)[1)]	1.92[a]	1.74[b]	1.88	1.91
tail, injury index (0-4)	0.42	0.89	0.28	0.75
tail, swelling index (0-1)	0.04	0.18	0.02	0.14
tail, blood spot index (0-1)	0.01	0.02	0.01	0.04
piglets (%) without tail cuts	67	36	84	40
piglets (%) with tail cuts <1/3	31	48	12	36
piglets (%) with tail cuts ≥ 1/3and < 2/3	2	16	3	19
piglets (%) with tail cuts ≥ 2/3	0	0	1	5

[1)] alfalfa pellets not included

Conclusion

Fiber rich feed, such as corn silage, alfalfa hay or grass pellets, fed in separate feed troughs did not depress feed intake or performance. Presence of fiber supplements often increased somewhat intake of concentrated feed, which resulted in poorer feed conversion ratios. Alfalfa pellets fed in separate feed troughs reduced tail biting to a significant extent but remained ineffective when being crushed and admixed to the diet. Other fiber rich feeds such as sugar beet pulp, corn silage or hay still wait to be tested for their potential impact on preventing tail biting

Apart from improving housing conditions and offering enrichment objects, feeding fiber rich feed can help suppressing tail biting, provided that these feed materials are offered separately and with an intact structure. In the construction of new stables, a second feeding line for fiber rich feed is already under discussion.

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Preis: (D) € 29,90

ISBN: 978-3-7690-4111-8