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- *Workshop-Beiträge*
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W. Windisch
Chairman

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Review lecture

Global perspectives and limits to livestock feeding

Niggli U. – Frick

The human population's diet is unsustainable and this has major implications for agriculture and livestock farming. This poses a risk to the stability of the planet, because several important indicators are reaching the carrying capacity of ecosystems (1). Ethical issues relating to meat consumption and animal welfare, the labelling of animal welfare programs and farm animal inspection are of great concern to the public and the media. Ways out are possible if the right framework conditions are in place. (Eco)efficiency, the predominant narrative of modern agricultural science, alone does not make agriculture sustainable. Sufficiency must complement it, which broadens the focus on food and nutrition. Livestock research can play an active role in finding solutions.

In 2050, an estimated 10 billion people are to be nourished with as little environmental damage as possible. Feeding today means a global average of 2850 kilocalories produced per capita and day (2) with a high proportion of animal proteins - and a disposal rate (food waste) of around 30 percent. Forecasts of FAO for 2050 even assume 3070 kilocalories per capita per day. Yet, the negative effects on the environment are increasing dramatically.

A study of FiBL (3) compared the current situation (base year) with the FAO reference scenario for 2050 and an alternative scenario of a drastic reduction in meat consumption, in which no more concentrated feedstuff is produced on arable land ("Food not Feed"). In all three scenarios modelled, 3.48 billion hectares of grassland were set. Arable land accounted for 1.54 billion hectares in the baseline scenario, 1.83 (+19 percent) in the FAO reference scenario for 2050, and 1.2 billion hectares (-22 percent) if the cultivation of concentrated feed for livestock is completely abandoned (which is of course an unrealistic scenario). While the FAO expects a strong increase in all farm animals, in the "Food not Feed" scenario the keeping of poultry and pigs was reduced dramatically (minus 70 respectively minus 88 percent), while all ruminant species increased slightly to strongly (plus 4 to 44 percent). All environmental indicators changed strongly positive in the "Food not Feed" scenario, both compared to the base year and to the FAO Reference Scenario 2050: less land under the plough, significantly lower N and P surpluses, less greenhouse gas emissions, less non-renewable energy, less pesticides, lower fresh water consumption, a decrease in deforestation and less water-induced soil erosion. The improvement in environmental impacts ranged from 19 to 46 percent. The average theoretical diet of people was kept stable and the same in all scenarios. For 2050, 3028 kilocalories (Kcal) per capita and day were available in the FAO reference scenario and 3008 Kcal in the "Food not Feed" scenario (for the base year, the figure was 2763 Kcal). The daily protein supply in all three scenarios was between 77 and 82 g protein per capita and day. The sources of energy and protein changed considerably: In the "Food not Feed" scenario, only 5 percent of the energy came from animal husbandry (in the FAO scenario it was 17 percent for 2050). For protein, the figure was only 11 percent (FAO scenario 38 percent). The majority of the protein came from plant products, as the cultivation of leguminous crops was expanded.

In a second study (4), FiBL examined different scenarios for future food and farming systems. If these are to be sustainable, trade-offs occur. For example, organic farming reduces nitrogen surpluses, protects soils and is less ecotoxic, but yields are lower. Or, grassland-based animal production does not compete for arable land with direct human nutrition, but emits more greenhouse gases per kilogram of meat than if the animals were to eat concentrated feed. When modelling the scenarios, the central question was therefore how to deal with these conflicting objectives.

The model calculations for 2050 showed that a massive conversion to organic farming used massively more arable land, if the consumer behavior remained the same - i.e. with a high proportion of animal products in the diet and large quantities of waste. This picture changed, however, if less concentrates were fed to livestock, people ate less meat and the amount of waste was reduced. An example: With 50 percent less concentrates, 50 percent less food waste and 60 percent organic farming, arable land consumption would hardly increase and the negative environmental impact would be greatly reduced.

In order to make agriculture sustainable, it is therefore necessary to look at the whole food system and not just at individual aspects such as farm production. Only by taking an overall perspective can the unavoidable conflicts of objectives be defused. In order to satisfy global hunger in a sustainable way, no radical solutions are needed, but a clever combination of efficiency, sensible use of resources (often referred to as consistency) and temperance (sufficiency). Then organic farming could play a central role in a sustainable food system of the future. In the study, organic farming was representative for any form of agriculture that greatly reduces environmental pollution (agro-ecological farming systems, Low External Input Sustainable Agriculture [LEISA] and also the strategies of ecological intensification).

Reducing food waste at least by 50 percent has also a huge economic significance. The value of the food thrown away amounts to one trillion US dollars per year, the burden on the environment costs 700 billion and the social costs amount to 900 billion. Together, the wasted food destroys 3 to 4 percent of the global gross national product (5).

The importance of animal husbandry for sustainable land use

On two-thirds of all land used for food worldwide, 3.4 billion hectares of permanent meadows and pastures, ploughing and thus arable farming is not possible. Admittedly, there are now techniques for bringing even marginal arable land into production. For example, the botanically species-rich savannah pastures in Brazil and Argentina were chemically "ploughed" with the total herbicide glyphosate, the nutrient-poor soils were fertilized and the genetically modified soya and maize varieties were planted without ploughs. The structurally unstable and shallow soils, in which no organic fertilizers are applied, are thus exposed to long-term destruction through erosion. Therefore, a continuous use of obligate grassland with ruminants is ecologically sustainable and for the sufficient global supply necessary. The grain growing on 390 million hectares of land used for feedstuff today could nourish four times as many people if directly fed to humans. Yet, this would not compensate for food from animal origin grown on 3.4 billion hectares of obligate grassland. People also often forgot that without livestock farming - yaks, cattle, buffalo, sheep, goats - for example, in the highlands of Nepal, the steppes of Mongolia, the Russian tundra, the African and Latin American savannah belts or the Alps, historically, there would be no humans.

Livestock farming is also important for functioning cycles of nutrients and organic material. Both in organic farming, where mixed farms are the rule, and in conventional production, where mixed farms are good agricultural practice, organic fertilizers can make a very high contribution to yield formation in arable farming. Because manure is important for keeping the organic soil matter content of arable fields stable (6) which can otherwise only be achieved with green manure for which without livestock, no value creation is possible.

Research needs for sustainable livestock husbandry

Eisler et al. (7) saw the greatest research needs for sustainable cattle farming in the following areas:

- Reduction of concentrated feed.
- Best forage production practice.
- Varied roughage with the possibility for the animals to choose feed materials (e.g. secondary plant metabolites).
- Breeding for life performance and roughage conversion.
- Low remounting rates.
- Healthy animals.
- Healthy young animals.
- Keeping an eye on costs and profits (veterinary costs and concentrated feed costs versus additional yield).
- Breeding efficient, robust and resistant animals.

Yet, the consumption of meat is a major concern for part of the society today. Therefore, completely new questions have to be answered by scientists. Are we allowed to keep 30 billion “fellow creatures” - from guinea fowl to buffalo - for the sole purpose of eating them after a short, often stressful life? The more we know about the behavior, social life, dexterity, and learning and combining abilities of animals, the more respect we have for them and the greater the resistance against killing. The once clear boundaries between animals and humans are increasingly disappearing. Can animals sense their fate? Do they know something about the end of their own existence? Do they feel affection or pain? Can they reflect about themselves? Can we still describe the behavior of humans and animals as being driven by reason or knowledge versus instinct? Many people doubt this. It will also be important to note that research and development of substitute meat and new foods will increase significantly.

Ways to a sustainable agriculture and animal welfare

Presumably, the current prices of organic products should correspond roughly to a realistic price for environmentally friendly production that also takes account of animal welfare. However, only the middle class can afford these prices. Therefore, the price for foods is part of the social policy of governments and this is at the expense of the environment. Therefore, various research teams are working on the theoretical foundations of ecological accounting ("true cost accounting"), which internalizes the environmental costs of any agriculture. This approach must be pursued further. It could be implemented relatively easily through levies on polluting substances such as nitrogen,

energy or pesticides. To change people's eating habits, it would make sense to shift environmental and energy costs upon animal feedstuff. This would make species-appropriate animal husbandry cheaper.

It would have an even greater leverage effect on sustainability if the EU and national governments could resolve the glaring contradictions between agricultural, environmental and health policies. Agriculture causes high repair costs to the environment, which in the case of drinking water are already costly for taxpayers. But climate change and biodiversity will make them even more expensive in the future. And cheap meat production is causing health costs to explode. It is therefore necessary to tax foods that are consistently high in fat and sugar. Eating unhealthy food should be really expensive, because otherwise the medical follow-up costs are enormously high. As the example of Denmark shows, such measures can only be introduced throughout Europe, otherwise consumers go shopping across the border. The current health and fitness trend might accelerate changes of eating habits. Comprehensive nutritional education at an early age is less costly to society than expensive repair medicine in old age.

The common agricultural policy also has the potential to direct €54 billion towards positive ecological and animal welfare impacts. The scientific community and extension services have developed methods and tools to analyze a farm within a few hours, how far it is from ecological, social and economic sustainability and how well the farm is managed. Therefore, holistic sustainability and impact assessment will become soon routine (8). In addition, the next CAP reform should finance programs for animal welfare, as Switzerland, for example, has been doing successfully for more than 20 years with the two programs RAUS (free-range husbandry and access to pastures) and BTS (particularly animal-friendly housing systems). This must be the goal of agricultural policy after 2020.

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FiBL Departement of Livestock Science, Ackerstrasse 113, 5070 Frick, Switzerland, urs.niggli@fibl.org

Abstracts

1.

In vitro effects of legumes with differing undegradable neutral detergent fiber concentrations supplemented with different carbohydrate sources 1: *In vitro* rumen fermentation parameters and microbial nitrogen production

In vitro Einfluss von Leguminosen mit unterschiedlichen unverdaulichen Neutrale-Detergenzienfasergehalten supplementiert mit unterschiedlichen Kohlenhydratquellen 1: In vitro Pansenfermentation und mikrobielle Stickstoffproduktion

*Nurdianti R. R., Pang C., Dickhoefer U., Castro-Montoya J. M. – Stuttgart

The aim of the present study was to evaluate the effects of different undegradable neutral detergent fiber (uNDF) concentrations of tropical legumes and a tropical grass incubated alone or combined and supplemented with different carbohydrate sources on rumen fermentation parameters and nitrogen (N) in rumen undegraded feed and liquid-associated microbes (LAM).

Methods: Two tropical legumes (i.e. *Vigna unguiculata* and *Centrosema pubescens*) and one tropical grass (i.e. *Pennisetum purpureum*) varying in uNDF concentrations were used for this study: low uNDF concentration (Vigna, 113 g/kg dry matter; DM), medium uNDF concentration (Pennisetum, 248 g/kg DM), and high uNDF concentration (Centrosema, 376 g/kg DM). Each legume and the grass were incubated alone or in legume+grass combinations (50:50) to create different diets varying in uNDF concentrations with forage concentrate ratio of 70:30. Diets were incubated along with either maize starch and sucrose as energy sources, and urea as N source to make the diets isonitrogenous (crude protein 221 g/kg DM). The energy source was weighed inside a filter bag (R510, ANKOM Technology, Macedon, USA; pore size = 50 ± 10 µm; length = 10 cm; width = 5 cm), while the forage portion was weighed directly into the incubation flask. The utilization of filter bags in an *in vitro* system allows for separation of the forage and concentrate fraction of a substrate without affecting the overall fermentation[1)]. In three runs, each diet was incubated in triplicate for 24 h in buffered rumen fluid[2)]. The ANKOM RF system (ANKOM Technology, Macedon, USA) was used to determine *in vitro* gas production (GP), volatile fatty acid (VFA), and ammonia-nitrogen (NH_3-N) concentrations as well as N concentration in the undegraded feed and LAM after 24-h incubation. All data were analyzed with a mixed model with forage and energy source as fixed effects and run as random effect. Significance level was $P < 0.05$.

Results: Vigna+Pennisetum had the highest GP (108 ml/g DM) among all forages, and sucrose had higher GP (96.9 ml/g DM) compared with maize starch (92.5 ml/g DM; $P < 0.01$) with no interaction effect between forages and energy sources ($P = 0.96$). Vigna+Pennisetum had the highest total VFA concentration in inoculum (61.0 µmol/ml) in all forages. Among all forages, the NH_3-N concentrations in the inoculum were the lowest at Pennisetum (178 mg/L; $P < 0.01$) and maize starch had higher NH_3-N concentration (149 mg/L) than sucrose (138 mg/L; $P < 0.01$). Minor differences in the N concentration of undegraded feed appeared between the legumes (5.16 vs. 5.08 mg/100 mg DM for Centrosema and Vigna, respectively) despite their different fiber characteristics. Interestingly, undegraded feed of substrates supplemented with maize starch had higher N concentration (4.68 mg/100 mg DM) than those with sucrose (4.40 mg/100 mg DM; $P < 0.01$). The N concentration of isolated LAM was the highest for the Pennsietum (8.50 mg/100 mg DM) followed by the grass combined with either legume (7.93-7.98 mg/100 mg DM), and the lowest for the legumes alone (7.89 and 7.74 mg/100 mg DM for Vigna and Centrosema, respectively). Sucrose had higher N concentration of LAM (8.05 mg/100 mg DM) than maize starch (7.96 mg/100 mg DM; $P < 0.01$). There was no interaction effect between forages and energy sources for N concentrations in undegraded feed ($P < 0.11$), yet an interaction was observed in N concentrations of LAM ($P < 0.01$).

Conclusions: The concentration of N in undegraded feed of legumes may not be affected by the uNDF concentration, but N supply via microbial mass seems to be lower with these forages.

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* *University of Hohenheim Animal Nutrition and Rangeland Management in the Tropics and Subtropics, Fruwirthstrasse 31, 70599 Stuttgart, Germany, risma_rizkia.nurdianti@uni-hohenheim.de*

2.

Effects of the conservation method of grass forages on nutritive properties of organically produced, raw cows' milk - fatty acid profile, vitamins B_1, B_2, B_6, B_{12} and E

Einfluss der Konservierungsmethode von Grünfutter auf die nutritiven Eigenschaften von ökologisch produzierter Milch - Fettsäuremuster, Vitamine B_1, B_2, B_6, B_{12} und E

*Haselmann A., Fuerst-Waltl B., Mayer H. K., Van den Oever S. P., Schmidt A., Schreiner M., Zebeli Q., Knaus W. – Vienna

"Hay milk" (i.e., from silage-free feeding) is of growing interest for dairy farmers in alpine regions because dairies pay higher prices for this milk. However, the conservation method of grass forages could have an effect on rumen fermentation and the resulting microbial formation of B vitamins and/or biohydrogenation of fatty acids. In addition, ensiled forages contain a significantly higher amount of α-tocopherol than dried (1). The objective of this study was to investigate the effects of the preservation method on the fatty acid profile and the concentration of vitamins (B_1, B_2, B_6, B_{12}, and E) in cows' milk.

Methods: Wilted forages (approx. 56% DM) of a grass-dominated sward (1st cut) were obtained from the same field and harvested at the same time (28 h after the cut). A single loading wagon picked up the windrows alternately, either for ensiling in a horizontal silo or barn-drying with a dehumidifier. A feeding trial with 18 Holstein cows started 9 month later. Cows were divided into 2 feeding groups by previous milk yield (MY; 30.0 ± 5.9 kg), body weight (717 ± 53 kg), DIM (171 ± 104 d), and parity (3.8 ± 2.1) into 2 feeding groups. Groups were either fed with silage (6.21 MJ NE_L, 124 g CP, 117 g WSC, and 477 g NDF per kg DM) or hay (6.15 MJ NE_L, 114 g CP, 180 g WSC, and 485 g NDF per kg DM). In addition, each cow received 3.6 kg (DM) of concentrate (7.52 MJ NE_L, 288 g CP, 131 g starch, and 251 g NDF per kg DM). Concentrate contained soybean cake, sugar beet pulp, wheat bran, molasses, and vitamins and minerals in a ratio (DM) of 51:23:18:3:5, respectively. Data collection started after a 2-week adaptation period of cows to the respective diet. On three days of the data collection period (d 1, 10, and 17), individual milk samples were taken which were derived from 2 consecutive milkings. For proximate analyzes, samples were pooled for each cow. The statistical model (proc glm SAS 9.4) included the treatment as fixed effect, and the MY as a covariable (only for vitamins). Interactions were only included in the model if $P \geq 0.10$.

Results: Arithmetic mean (± SD) of daily MY (20 d) was 28.1 ± 7.05 and 29.4 ± 6.80 kg for silage and hay group, respectively. There was no treatment effect on the proportion of *de novo*, saturated, mono-unsaturated, and odd and branched chain fatty acids in milk fat. As compared to silage, hay feeding increased proportion of poly-unsaturated fatty acids (+ 0.53 g / 100g milk fat; $P = 0.002$) due to elevated amounts of linoleic acid (+ 0.3 g; $P = 0.005$) and α linolenic acid (+ 0.31 g; $P < 0.001$).

No effects could be detected for the total concentration of vitamin B_6 in milk. Vitamin B_2 (1.71 ± 0.287 mg/L) tended to be higher (+0.25 mg/L; $P = 0.073$) in the milk of silage-fed cows as opposed to hay-fed cows. Vitamin B_1 (0.27 ± 0.056 mg/L) and B_{12} (3.16 ± 1.109 µg/L) were also positively affected by silage feeding ($P = 0.032$ and 0.024, respectively) but the effects were lessened with increasing MY of cows (> 30 kg/d; interaction, $P = 0.036$ and 0.028, respectively). Although we found an interaction ($P = 0.096$) for vitamin E, the content was 1.9-fold higher (1.15 vs. 0.62 mg/L) in the milk of silage-fed cows, as compared to hay-fed cows, when calculating for the average MY of each group.

Conclusions: The forage conservation method impacted nutritionally relevant parameters of raw cow's milk. While silage enhanced the content of vitamin B_{12} and E at the average milk production level in the current trial, hay positively modulated the fatty acid profile towards higher proportions of linoleic and α-linolenic acid, regardless of the MY. Given the relevance of high-producing dairy cows, it would be interesting to study possible effects on milk quality when using diets with higher concentrate levels.

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* *University of Natural Resources and Life Sciences (BOKU) Department of Sustainable Agricultural Systems, Gregor Mendel Strasse 33, 1180 Vienna, Austria, andreas.haselmann@boku.ac.at*

3.

The effect of insect meal from *Tenebrio molitor* L. on the metabolism of growing pigs

Wirkung von Insektenmehl aus Tenebrio molitor L. auf den Stoffwechsel wachsender Schweine

*Ringseis R., Meyer S., Braune M., Friedhoff T., Most E., Liebisch G., Zorn H., Geßner D. K., Eder K. – Giessen / Regensburg

Owing to the growing demand for protein as feed and the increasing pressure on limited natural resources, there is an urgent need for alternative protein sources which can be efficiently produced with lower environmental impact. In this regard, protein-rich insect meal (IM) may offer great potential, because it can be produced from industrialized mass-rearing of edible insects, such as *Tenebrio molitor* L., with higher feed conversion ratios and lower utilization of natural resources than conventional protein sources, such as soybean meal (SM). One important prerequisite for the use of IM as feed for farm animals is that it does not cause adverse effects on intermediary metabolism. While some recent studies demonstrated that inclusion of IM in feeding rations for broilers and pigs neither induces detrimental effects on blood chemical parameters nor causes any histopathological alterations, any in-depth investigations on the metabolic effects of IM in pigs were completely lacking. Against this background, the present study aimed to comprehensively describe the effects of IM on intermediary metabolism of pigs by means of omics-techniques.

Methods: 5-week-old crossbred pigs were randomly assigned to 3 groups (CON, IM5, IM10) of 10 pigs each with similar body weights (BW). Three isocaloric and isonitrogenous diets sufficient to meet the requirements according to GfE (2006), but differing in their main protein source were fed. In the diet of group CON, which contained SM as the main protein source, the amount of IM from *Tenebrio molitor* L. was 0%, while in the diets of groups IM5 and IM10 the amount of IM was 5 and 10%, respectively, through partial (50%) or complete (100%) isonitrogenous replacement of SM (44% crude protein) by IM. After feeding the diets for 4 weeks, the pigs were killed and key metabolic tissues (liver, muscle, plasma) were analyzed using transcriptomics, metabolomics and lipidomics. Normally and not-normally distributed data were analyzed by one-way ANOVA and Kruskal-Wallis test, respectively.

Results: Performance parameters (daily BW gain, daily feed intake, gain:feed-ratio) did not differ across the groups, despite ileal digestibilities of most amino acids were 6.7 to 15.6%-units lower in IM10 than in CON ($P < 0.05$). Transcriptomics of liver and skeletal muscle revealed a total of 166 and 198, respectively, transcripts differentially expressed between IM10 and CON. Plasma metabolomics revealed higher concentrations of alanine, citrulline, glutamate, proline, serine, tyrosine and valine and a lower concentration of asparagine in IM10 than in CON ($P < 0.05$). Only one out of fourteen quantifiable biogenic amines, namely methionine-sulfoxide (MetS), in plasma was elevated by 45 and 71% in IM5 and IM10, respectively, compared to CON ($P < 0.05$). Plasma concentrations of both, major carnitine/acylcarnitine species and bile acids were not different across groups. Lipidomics of liver and plasma demonstrated no differences in the concentrations of triacylglycerols, cholesterol and the main phospholipids, lysophospholipids and sphingolipids between groups. The percentages of all individual phosphatidylcholine (PC) and phosphatidylethanolamine (PE) species in the liver showed no differences between groups, except those with 6 double bonds (PC 38:6, PC 40:6, PE 38:6, PE 40:6), which were markedly lower in IM10 than in CON ($P < 0.05$). In line with this, the percentage of C22:6n-3 in hepatic total lipids was lower in IM10 than in the other groups ($P < 0.05$).

Conclusions: Comprehensive analyses of the transcriptome, lipidome and metabolome of key metabolic tissues indicate that the use of IM as the main protein source in the diet has only a weak impact on the intermediary metabolism of growing pigs. Thus, IM from *Tenebrio molitor* L. can be considered as a safe dietary protein source for growing pigs.

* *Justus-Liebig-University Giessen Institute of Animal Nutrition and Nutrition Physiology, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany, robert.ringseis@ernaehrung.uni-giessen.de*

4.

Effect of feeding a diet containing 10% lignocellulose on the gastrointestinal tract development, intestinal microbiota and excreta characteristics of dual purpose laying hens

Effekte der Fütterung eines mit 10 % Lignocellulose versetzten Futters auf die Entwicklung des Gastrointestinaltraktes, die intestinale Mikrobiota und Exkrementbeschaffenheit von Zweinutzungshennen

*Röhe I., Vahjen W., Metzger F., Zentek J. – Berlin

Lignocellulose is a constituent of plant cell walls and can be considered as a diet diluent. Studies suggest that feeding lignocellulose at low inclusion levels stimulate digestive tract development (1) and modulate intestinal microbiota and metabolites in chickens (2). Moreover, it could be demonstrated that dietary lignocellulose at low inclusion levels might have a beneficial effect on litter quality (2). The aim of the present study was to investigate the impact of feeding diets containing higher levels of lignocellulose on the development of gastrointestinal organs, intestinal microbiota and excreta characteristics in dual purpose laying hens.

Methods: One-day-old female Lohmann Dual chicks were allocated to 12 pens and fed two different diets resulting in six replicates per feeding group: the basal control diet (CON) and a treatment diet (LC), based on the control diet but diluted with 10% lignocellulose (ARBOCEL® R, J. Rettenmaier & Söhne GmbH + Co KG, Germany). At 52 weeks of age, gastrointestinal organs were weight and tissue samples taken for histomorphological examinations. Caecal digesta samples were analysed for bacterial metabolites and composition using gas chromatography, HPLC, photometry, and PCR. Excreta dry matter and viscosity was consistently assessed during the trial. Statistical analyses were performed using Students t test and Spearman correlation analyses (SPSS 25.0, Chicago, IL).

Results: LC-fed hens showed increased weights of the gizzard, small and large intestine ($P < 0.05$) compared to hens fed CON. LC-fed hens had a larger colorectal villus area and mucosal enlargement factor than CON fed hens ($P<0.05$). The concentration of short-chain fatty acids (SCFAs) and ammonia was higher in CON-fed hens compared to LC-fed hens. Bacterial composition and activity was not affected by feeding the different diets. The concentration of SCFAs in the caecum was negatively correlated with the colorectal villus surface area ($P < 0.05$). LC-fed hens had a higher excreta dry matter content than hens fed CON at 10, 17 and 22 weeks of age ($P<0.05$).

Conclusions: The results of this study showed that feeding of higher levels of lignocellulose increased gastrointestinal organ weights and stimulated colorectal mucosal development of dual purpose hens. A higher intestinal surface area in combination with lower concentrations of SCFAs suggest a compensatory reaction of hens fed LC enhancing the absorption of bacterial metabolites by increasing the intestinal mucosal surface. This hypothesis is supported by analyses of caecal microbiota demonstrating that microbial composition and activity was not affected by feeding the diets. Furthermore, the inclusion of dietary lignocellulose generally decreased the excreta moisture content, which might have positive effects on litter quality under practical conditions.

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* *Freie Universität Berlin Institute of animal nutrition, Königin-Luise Straße 49, 14195 Berlin, Germany, ilen.roehe@fu-berlin.de*

5.

Effect of earthworm or vermicompost feeding in early life on growth performance and faecal consistency of broiler chicks

Auswirkungen der Fütterung von Regenwürmern oder Wurmkompost ab dem ersten Lebenstag auf Wachstumsleistung und Fäkalkonsistenz von Masthühnchen

*Das G., Seyedalmossavi M. S. M., Li Z., Troescher A., Metges C. C. – Dummerstorf / Mannheim

Fast-growing broilers have an impaired ability to cope with challenges (1). Development of the immune system is promoted by an early exposure to microorganisms, which is essential for the control of pathogens (2). Broiler chicks hatch in incubators, and are reared under highly hygienic conditions, implying the lack of a proper exposure to microorganisms. Earthworms (EW) belong to natural feed sources of chickens. Since EW are dependent on microorganisms for digestion of cell wall constituents of plants, and have a high microbial activity in their gut (3), they may be considered as 'natural' microbiota inoculates for chicks. We hypothesized that feeding of EW or vermicompost (VC) to broiler chicks in the early phase of life provides beneficial effects on performance and gut health, and helps cope with environmental stressors (i.e. feed change).

Methods: A total of 480 male Cobb-500 birds were used in a feeding trial with two identical runs. In each run, 240 birds were housed in 24 pens (n=10 birds/pen). Per run and starting from the first day (d) of life onwards, the birds were fed either a control diet (CON+, n=120) or CON+ supplemented with either 1% EW (on dry matter basis, DM) (CON+EW; n=60) or VC (CON+VC; n=60) for 8 d (Period 1; P1). On d-8, half the birds in each group were sampled for digesta and intestinal size measurements. Half the remaining birds on CON+ diet (n=30) were either kept on the same diet for further 8 d (P2) or given another diet that replaced approximately 50% of corn in the CON+ with wheat, barley and rye to produce a challenge diet with higher non-starch polysaccharides (NSP) (i.e. negative control diet, CON-, n=30). The birds consuming EW and VC in P1 were fed the CON- diet in P2 (i.e., CON-EW and CON-VC, respectively). All 4 diets had similar energy (ca. 12.4 MJ/kg) and protein (220 g CP/ kg) contents. On d-16, all the remaining birds were killed. Pen based average body weight (BW), daily weight gain (ADG), feed intake (FI), DM intake (DMI), and feed conversion ratio (FCR) were calculated in the end of each P. In the end of P2, the birds were evaluated for the presence of sticky faeces (SF) attached to cloaca. Data were analyzed using the GLM procedure of SAS (V9.4).

Results: Overall mortality was low (ca. 2 %). Litter DM did not differ among 4 diets in either period ($P>0.05$). CON+VC improved ($P<0.05$) BW and ADG in P1 through an elevated feed intake ($P<0.05$), but had no effect on FCR ($P>0.05$). CON+EW did not differ from the CON+ in terms of growth and feed intake ($P>0.05$) in P1. In P2 CON- did not affect growth and DMI relative to CON+ ($P>0.05$). In P2, CON-VC fed birds were still heavier than those fed on CON+. However, ADG of birds on CON-VC did not differ from those birds on other diets ($P>0.05$). With CON+ diet (P1), prevalence of SF was negligible (2.6%). In the end of P2, 10% of CON+ birds had SF. CON- ($P<0.05$) increased prevalence of SF (40.5%), and VC aggravated this effect (57.9%), whereas CON-EW (18.9%) did not differ from CON+ ($P>0.05$). As compared with CON-, CON-EW tended to decrease ($P=0.072$) prevalence of SF. Birds fed on CON-EW had heavier caeca than those birds fed on CON+ ($P<0.05$).

Conclusions: EW (1%) induced no negative effect on performance. VC (1%) increased feed intake and growth in the first week of life. CON- did not impair performance but increased prevalence of SF, likely due to increased viscosity induced by NSP. The lower incidence of SF due to EW may be indicative of a successful inoculation with beneficial microorganisms that might reduce anti-nutritive effects of NSP.

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* *Leibniz Institute for Farm Animal Biology Institute of Nutritional Physiology, 18196 Dummerstorf, Germany, gdas@fbn-dummerstorf.de*

6.

In vitro effects of legumes with differing undegradable neutral detergent fiber concentrations supplemented with different carbohydrate sources 2: Enzymatic intestinal digestibility of rumen undegraded feed and microbial mass

In vitro Einfluss von Leguminosen mit unterschiedlichen unverdaulichen Neutrale-Detergenzienfasergehalten supplementiert mit unterschiedlichen Kohlenhydratquellen 2: Enzymatische intestinale Verdaulichkeit von im Pansen nicht abgebautem Substrat und mikrobielle Masse

*Nurdianti R. R., Pang C., Dickhoefer U., Castro-Montoya J. M. – Stuttgart

The aim of the present study was to evaluate the effects of different undegradable neutral detergent fiber (uNDF) concentrations of tropical legumes and tropical grass incubated at different proportions supplemented with different carbohydrate sources on *in vitro* enzymatic intestinal digestibility i.e. intestinal dry matter digestibility (iDMD) and intestinal crude protein digestibility (iCPD) of undegraded feed and liquid-associated microbes (LAM).

Methods: Two tropical legumes (i.e. *Vigna unguiculata* and *Centrosema pubescens*) and one tropical grass (i.e. *Pennisetum purpureum*) varying in uNDF concentrations were used for this study: low uNDF concentration (Vigna, 113 g/kg dry matter; DM), medium uNDF concentration (Pennisetum, 248 g/kg DM), and high uNDF concentration (Centrosema, 376 g/kg DM). Each legume and the grass were incubated alone or in legume+grass combinations (50:50) to create different diets varying in uNDF concentrations with forage concentrate ratio of 70:30. Diets were incubated along with either maize starch and sucrose as energy sources, and urea as N source to make the diets isonitrogenous (crude protein 221 g/kg DM). The energy source was weighed inside a filter bag (R510, ANKOM Technology, USA; pore size = 50 ± 10 µm; length = 10 cm; width = 5 cm), while the forage portion was weighed directly into the incubation flask. The utilization of filter bags in an *in vitro* system allows for separation of the forage and concentrate fraction of a substrate without affecting the overall fermentation[1)]. In three runs, each diet was incubated in triplicate for 24 h in buffered rumen fluid[2)] using ANKOM RF gas production system (ANKOM Technology, USA). After 24–h incubation, the filter bag of each flask was transferred to polyethylene bottles and centrifuged at 500 *g* for 10 min at 4 °C (Hettich Rotanta RPC, Andreas Hettich GmbH & Co. KG, Germany). The supernatant was transferred into Beckman polycarbonate 250–mL bottle and centrifuged at 20,000 *g* at 4 °C for 8 min (Beckman Avanti J25 centrifuge, Rotor JA14, GMI, USA) to obtain the undegraded feed and LAM fractions. The undegraded feed and LAM samples were incubated using *in vitro* enzymatic intestinal digestibility procedure in order to determine the iDMD and iCPD of undegraded feed and LAM[3)]. All data were analyzed with a mixed model with forage and energy source as fixed effects and run as random effect. Significance level was $P < 0.05$.

Results: Among all forages, Vigna (the lowest uNDF concentration) had the highest iDMD of undegraded feed (61.2 g/100 g) and the highest iDMD of LAM (92.0 g/100 g). Additionally, sucrose had higher iDMD of undegraded feed (46.9 g/100 g) and LAM (86.5 g/100 g) compared to maize starch (iDMD of undegraded feed; 45.3 g/100 g, iDMD of LAM; 84.5 g/100 g, $P < 0.01$). Regarding iCPD of undegraded feed, Pennisetum had the highest (75.9 g/100g) followed by Vigna+Pennisetum (75.4 g/100 g), Vigna (72.2 g/100 g), Centrosema+Pennisetum (70.1 g/100 g), and Centrosema (66.6 g/100 g) subsequently. Maize starch in iCPD of undegraded feed (72.8 g/100 g) was higher than sucrose (71.3 g/100 g; $P< 0.01$). Regarding iCPD of LAM, Pennisetum was also the highest (85.8 g/100 g) followed by Vigna (84.9 g/100 g), Centrosema+Pennisetum (81.6 g/100 g), Centrosema (79.6 g/100 g), and Vigna+Pennisetum (79.5 g/100 g) respectively. Sucrose had higher iCPD of LAM (83.5 g/100g) than maize starch (81.0 g/100 g; $P < 0.01$). Additionally, the interaction between uNDF concentrations and energy sources was not observed in any iDMD and iCPD of undegraded feed and LAM ($P > 0.05$).

Conclusions: Enzymatic degradability of DM and CP from the rumen outflow appears to be hampered by high uNDF concentrations in the legumes, thus decreasing the supply of absorbable nutrients. However, this must be related to the total amount of undegraded feed and LAM produced and flowing from the rumen to the intestine.

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* *University of Hohenheim Animal Nutrition and Rangeland Management in the Tropics and Subtropics, Fruwirthstrasse 31, 70599 Stuttgart, Germany, risma_rizkia.nurdianti@uni-hohenheim.de*

7.

Effect of different herbage conservation methods on milk composition and milk sensory properties

Effekt unterschiedlicher Konservierungsverfahren von Wiesenfutter auf die Zusammensetzung der Milch und ihre sensorischen Eigenschaften

*Manzocchi E., Giller K., Bord C., Verdier-Metz I., Bouchon M., Berard J., Kreuzer M., Martin B., Coppa M. – Zurich / Lempdes / Saint Genès-Champanelle / Lindau / Marcenat / Aurillac

The nutritional benefits of milk produced in grass-based dairy systems (e.g. richer in omega-3 fatty acids, fat-soluble vitamins and antioxidants) are well known. However, different methods of herbage conservation (drying and ensiling) and feeding (grazing and indoor feeding of fresh herbage) also affect the milk properties. Recently, milk and milk products from cows fed no fermented forages was recognized as a guaranteed traditional speciality (g.t.S.) by the European Union and sold under a 'hay milk' label. The increasing interest of consumers for authentic and high-quality products reveals the need for a reliable differentiation between silage- and silage-free produced milk. This study investigates the effects of the herbage conservation method as well as grazing and indoor grass feeding on composition and sensory quality of the milk.

Methods: Grass for hay and silage production was harvested on the same day from the same plot in May 2019. Forty-eight late lactating dairy cows (24 Holstein, 24 Montbéliarde) were allocated to four balanced groups. One group was fed hay, the second group was fed grass silage. The third and fourth groups were fed fresh herbage from the regrowth of the same plot previously used for hay and silage making either indoors or by strip-grazing. All animals were supplemented with 3 kg/day of concentrate and had free access to mineral blocks and water. Fifteen days of adaption were followed by 10 days of sampling. During the sampling period, milk yield was registered daily and individual milk samples were collected twice a week from morning and evening milkings. Each group was divided into three balanced sub-groups (two Holstein, two Montbéliarde). Bulk milk samples from evening and morning milkings of each sub-group were collected once during the sampling period. Bulk milk samples of the subgroups were pasteurised and conserved at 4°C for sensory evaluation on the following day. Nine trained panellists performed a descriptive sensory analysis (19 descriptors of colour (1), odour (5), taste (6), aroma (5), and texture (2); graded from 0 to 10) of the 12 milks, which were served in a sequential monadic way at room temperature (20±2°C). Individual milk samples were analysed by infrared spectroscopy (Milkoscan 6000, Foss, Hilleroed, Denmark) for contents of fat, protein, lactose, casein and urea. Data on milk yield and composition were analysed with PROC MIXED in SAS. Milk sensory analysis data were analysed with SensoMineR in R.

Results: The milk yield did not differ between the treatments with 13.8 ± 3.04 kg/day (mean ± standard deviation). Likewise, the milk fat content did not differ between the four treatment groups and was on average 36.9 ± 3.95 g/L. The protein content was higher ($P=0.03$) in the milk of the hay-fed cows (33.6 g/L) in comparison to the cows fed silage (30.7 g/L) and fresh herbage indoor (30.6 g/L). The protein content in milk from grazing cows (31.5 g/L) did not differ from the other treatments. The milk urea content was higher ($P<0.001$) in the milk of silage-fed (294 mg/L) and grazing cows (288 mg/L), than in the milk of cows fed hay (206 mg/L) or fresh grass indoor (220 mg/L). Milk from hay-fed and silage-fed cows had a less intense colour than that from cows fed fresh herbage indoors or on pasture. The milk from hay-fed cows had a less intense odour than the milk from grazing cows. Furthermore, creamy odour and creamy aroma were less intense in the milk from hay-fed cows than in all other milks. Moreover, milk from hay-fed cows had a less intense 'cooked milk' odour than milk from cows fed fresh herbage indoor.

Conclusions: The herbage conservation and feeding method had a clear effect on the milk's sensory properties. This was most pronounced with milk from fresh herbage, and less clear with silage-based milk, only partially justifying the aforementioned labelling from a sensory perspective.

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* *ETH Zurich Institute of Agricultural Science, Universitaetstrasse 2, 8092 Zurich, Switzerland, elisa.manzocchi@usys.ethz.ch*

8.

The effect of insect meal from *Tenebrio molitor* L. on the gut microbiome and parameters of intestinal mucosa function of growing pigs

Wirkung von Insektenmehl aus Tenebrio molitor L. auf das Darmmikrobiom und Parameter der Intestinalmukosafunktion wachsender Schweine

*Ringseis R., Meyer S., Röhrig J., Most E., Zorn H., Geßner D. K., Eder K. – Giessen

Insect meal (IM) obtained from mass-rearing of edible insects, such as *Tenebrio molitor* L., is considered as a promising alternative protein source with lower environmental impact than conventional protein sources, such as soybean meal (SM). Apart from overcoming currently existing legal obstacles, a key prerequisite for the inclusion of IM in feeding rations for monogastric farm animals is that animal´s health and performance is not impaired. While a great number of studies in broilers indicate that IM is a suitable dietary protein source in this regard, too few studies are available in pigs to reliably assess the suitability of IM as protein source in feeding rations for growing pigs. In view of this, the present study aimed to study the effect of IM on the gut microbiome and parameters of gut health which is vital to both, overall health and performance.

Methods: Five-week-old crossbred pigs (n = 30) were randomly assigned to three groups of 10 pigs each with similar body weights (BW) and fed isocaloric and isonitrogenous diets either without (group CON) or with 5% IM (group IM5) or 10% IM (group IM10) from *Tenebrio molitor* L. for 4 weeks. Isocaloric replacement of SM by IM was achieved by inclusion of 2.8% and 5.7% cellulose in the IM5 and IM10 diets, respectively. Concentrations of crude fiber were 6.3, 8.0 and 9.7% in the CON, IM5 and IM10 diets, respectively. At the end, pigs were killed and cecal digesta was collected to analyse the microbial community by 16S rRNA sequencing and to determine the concentrations of important microbial fermentation products [short-chain fatty acids (SCFAs)]. Mucosa samples were taken from small intestine and mRNA levels of genes encoding nutrient transporters, inflammatory mediators, tight junction proteins and mucins were determined as parameters of intestinal mucosa function by qPCR. Normally and not-normally distributed data were analyzed by one-way ANOVA and Kruskal-Wallis test, respectively.

Results: All performance parameters investigated, such as final body weights, daily body weight gain, daily feed intake and gain:feed-ratio, of the pigs did not differ between the three groups. The relative abundance of *Bacteroidetes*, the most abundant phylum in cecal digesta of all groups, was lower in group IM10 than in group CON ($P <$ 0.05). Relative abundance of *Firmicutes*, the second most abundant phylum, and the *Firmicutes*:*Bacteroidetes*-ratio tended to be higher in groups IM10 and IM5 than in group CON ($P < 0.1$). The relative abundance of the third most abundant phylum *Proteobacteria* tended to be higher in group IM10 than in groups CON and IM5 ($P < 0.1$). Relative abundance of the phylum *Spirochaetes* in cecal digesta was strongly reduced in group IM10 compared to group CON ($P < 0.05$), whereas that of *Actinobacteria* was higher in group IM10 than in group CON ($P < 0.05$). Concentrations of total SCFAs and main individual SCFAs (acetic acid, propionic acid, butyric acid) in cecal digesta did not differ between the three groups, whereas concentrations of minor SCFAs (isobutyric acid, isovaleric acid, valeric acid) in cecal digesta were higher in group IM5 and IM10 than in group CON ($P < 0.05$). The mRNA levels of genes encoding transporters for carbohydrates and peptides in duodenal and jejunal mucosa did not differ between groups. While the mRNA levels of genes encoding tight junction proteins and mucins in ileal mucosa did not differ between the three groups, the mRNA level of the inflammatory gene tumour necrosis factor was lower in groups IM5 and IM10 than in group CON ($P < 0.05$) and that of interleukin-6 tended to be lower in group IM10 than in group CON ($P < 0.1$).

Conclusions: IM in the diet of growing pigs causes significant changes in the gut microbiome. Considering that performance parameters did not differ across the groups of pigs, the IM-induced changes of the gut microbiome do not argue against the use of IM from *Tenebrio molitor* L. as a dietary protein source for growing pigs.

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* *Justus Liebig University Giessen Institute of Animal Nutrition and Nutrition Physiology, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany, robert.ringseis@ernaehrung.uni-giessen.de*

9.

Tenebrio molitor press cake as an alternative protein source for piglets

Presskuchen aus Larven des Mehlkäfers (Tenebrio molitor) als alternative Proteinquelle für Ferkel

*Sünder A., Brede A., Wecke C., Liebert F. – Göttingen

One of the potential alternative protein sources for the substitution of soybean meal (SBM) is insect meal from yellow mealworm *Tenebrio molitor* (TM). The current study aimed to evaluate the effects of partial replacements of SBM by a press cake from TM as protein source (25%, 50% and 75%) on zoo-technical data of weaned piglets.
Methods: 40 weaning piglets (male, castrated) were randomly allotted to four diets. Piglets with 6.7 ± 0.9 kg body weight at the beginning were individually housed in flat deck cages for 41 days (13 days pre-period; 28 days growth trial). The pre-period was necessary to adapt the freshly weaned piglets to the new diet and to achieve a stable dry matter intake. The diets were fed *ad libitum* but divided into two servings per day to minimize spilling of feed. Refusals were collected and recorded. Feed intake and body weight were recorded weekly.
The control diet was based on wheat, barley, soybean oil and 28.7 % SBM as protein source. In the three experimental diets, the SBM was substituted by 25%, 50% and 75% partly defatted TM press cake (75 % crude protein (CP) in DM) resulting of isonitrogenous diets (21.4 ± 0.2 % CP in DM). As TM press cake contains more crude fat than SBM (6.8% versus 2.1%) the soybean oil content of the experimental diets was reduced to realize isoenergetic rations (15.6 MJ/kg DM). According to results of earlier experiments with alternative protein sources (partly defatted meal of *Hermetia illucens* and algae meal of *Spirulina platensis* by piglets or TM press cake by growing chicken), underlining the importance of adjusted amino acid supply (1, 2, 3), actually lysine, threonine and methionine were added depending on the TM press cake content in the diets.
Results were statistically evaluated by one-way ANOVA (IBM SPSS Statistics, Version 26) to identify significant differences ($p < 0.05$).
Results: The results of the growth study did not show any significant differences regarding the final body weight (22.6 ± 0.4 kg), daily gains (442 ± 9 g/d), feed consumption (592 ± 17 g/d) and feed conversion ratio (1.34 ± 0.03 g/g). Only feed intake tended to be higher with increasing protein from TM press cake (Control: 567 g/d, 25% TM: 593 g/d, 50% TM: 601 g/d and 75% TM: 605 g/d) resulting in a numerically higher FCR of the 75% TM group compared to the three other groups (1.39 g/g versus 1.33 g/g).
Conclusions: The current investigation indicated satisfying palatability and acceptance of diets containing TM press cake. Replacement of SBM by TM press cake as protein source up to 75% was possible without negative effects on performance data, if the amino acid profile is adjusted by the use of isolated amino acids as recommended regarding requirements and ratio to lysine.

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* *Georg-August-Universität Göttingen DNTW / Tierernährungsphysiologie, Kellnerweg 6, 37077 Göttingen, Germany, asuende@gwdg.de*

10.

In vitro studies on gas production from wheat or rye – and by-products (bran and dried distillers grains with solubles - DDGS) – during fermentation using microflora of ileal digesta and faeces from Göttingen minipigs

In vitro Studien zur Gasproduktion von Weizen oder Roggen - und deren Nebenprodukten (Kleie/Trockenschlempe - DDGS) - bei Fermentation mit Mikroflora aus Ileumchymus und Kot von Göttinger Miniaturschweinen

*Hartung C. B., Grone R., Kamphues J. – Hanover / Bergen

Rye has only played a minor role in swine feeding for a long time for various reasons but new findings indicate potential health promoting effects of this cereal due to its high contents of dietary fiber and its higher fermentability in the large intestine [1].The aim of this study was to compare gas production as a sign of fermentability of rye- and wheat-products in vitro with the flora of ileal digesta and faeces from Göttingen minipigs as inoculum.

Methods: Two adult Göttingen minipigs (32.5 ± 0.56 kg; 6.5 ± 1.0 years) were fed a test diet to adapt their gut microflora to the above mentioned substrates (diet containing 27% wheat, 25% rye, 10% soybean-meal, 10% rapeseed-meal). The individually housed pigs were fitted with an ileo-caecal fistula. After one week of adaptation one month of collection followed to obtain enough ileal digesta and faeces for six repetitions of each fermentation with each substrate (three repetitions with digesta/faeces of each pig). During this time fresh faeces was collected and frozen at -22°C directly. Twice weekly the fistula was opened directly after feeding and for the next 6 hours ileal digesta was collected and frozen at - 22°C directly as well (preliminary tests showed a delayed gas production but comparable final gas pressure values when frozen versus fresh material was used). The collected material was used as inoculum for the fermentation later after thawing at 8°C over night under anaerobic conditions (CO_2). For the fermentation 30g of the defrosted inoculum was mixed with 150ml of buffer solution [2] with a hand blender under CO_2 gas supply. The mixture was filtered through a 200µm-sieve and 110ml of the suspension was mixed with 440ml of buffer solution. After this preparation, 100ml of the suspension was given into a bottle with 1.0g of substrate (either ground rye/wheat, rye-/wheat-bran or rye-/wheat-DDGS) and incubated at 38°C for 12h (ileal digesta) or 24h (faeces). Gas production was measured as cumulative pressure in mbar forming during fermentation using the ANKOM RF Gas Production System. Statistical analyses were performed using the SAS® software (ANOVA).

Results: With ileal digesta as inoculum the gas pressure values were only numerically higher for the rye by-products than for the wheat by-products and ground wheat reached slightly higher values than ground rye (2739 ± 600 mbar and 2686 ± 897 mbar respectively). Rye and its by-products passed the mark of 500 mbar at least one hour earlier than respective wheat-products in all approaches (except ground wheat and rye fermented with ileal digesta; both after 9 hours). Fermentations with faeces yielded in higher gas pressure than with ileal digesta, most obviously when using DDGS as a substrate (486 ± 141 mbar wheat/519 ± 190 mbar rye with ileal digesta and 1696 ± 216 mbar wheat/2480 ± 253 mbar rye with feces). With faeces as inoculum rye and its by-products finally yielded in significantly ($p<0.05$) higher gas pressure than wheat and the respective by-products. The pH-values in the approaches decreased similarly (larger decrease for rye and by-products than for wheat and by-products; rye with faeces 1.14 ± 0.383 units, rye with ileal digesta 1.13 ± 0.545 units, wheat with faeces 0.932 ± 0.464 units, wheat with ileal digesta 1.02 ± 0.627 units).

Conclusions: As gas production and acidification display bacterial activity and were higher for rye and its by-products, it can be concluded that those products provide more fermentable substrate for the microflora, especially in the hindgut ("feed" for the hindgut) with corresponding positive effects [3]. DDGS has to be named in particular as it contains hardly any starch and resulted in very low gas pressure when fermented with ileal digesta thus reaching the hindgut more or less undegraded.

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* *University of Veterinary Medicine Hannover, Foundation Institute for Animal Nutrition, Bischofsholer Damm 15, 30173 Hanover, Germany, clara.berenike.hartung@tiho-hannover.de*

11.

The larvae of the Black Soldier Fly: a suitable source of protein and fat for laying hens?

Sind die Larven der schwarzen Waffenfliege eine geeignete Protein- und Fettquelle für Legehennen?

*Heuel M., Sandrock C., Mathys A., Gold M., Zurbrügg C., Kreuzer M., Terranova M. – Zurich / Frick / Dübendorf

The global demand for soybean as a feed ingredient is high, but its use is controversially discussed in Europe (1). One of the options for replacing soybean being currently researched is insect protein meal. So far, in Europe, insect meal is not allowed as an ingredient of poultry diets. However, since meal from some insect species is already approved for pets, aquaculture and even human consumption, an approval for poultry is expected soon. For animal nutrition, especially the larvae of the black soldier fly (BSF, *Hermetia illucens*) are considered promising because they can be reared locally e.g. on former food products and the protein is rich in lysine and methionine (2). Still it is not yet known, if BSF meal can fully replace soybean protein and if the larval fat, prevalent in the larvae, would be suitable to be fed to poultry, too. Therefore, the replacement with insect material was tested in high yielding layers fed an organic diet type.

Methods: For a 7-week period, 50 Lohmann Brown Classic hens in week 7 of lay were allocated to five dietary treatments in a randomised design. The control diet was based on 15% soybean cake and 3% soybean oil (SS). The four experimental diets built on nearly the same proportions of two defatted BSF larval meals and two larval fats of two different origins (A and B), namely (15% meal/2% fat) were AA, AB and BB and AS. Larvae of type A had been grown on a mixture of wheat bran, French fries and cereal mill offals, type B larvae had received a mixture of fruit and vegetable raw waste, brewer's grain and pasta production waste (descending order). Diets were balanced for fat and protein contents, where, due to the high residual fat content in the larval meal B, no extra fat was added to diet BB. The birds were kept individually in enriched cages (80×80×80 cm). Laying performance and egg weight were measured daily. Body weight and feed intake were determined weekly. During the experiment, six eggs per hen were sampled on consecutive days to analyse shell breaking strength, yolk colour, Haugh units, proportions of shell, yolk and egg and chemical composition of yolk and egg white, the latter after lyophilisation and grinding. Data were evaluated by the Mixed procedure of SAS (version 9.4) with diet as fixed effect and hen as experimental unit.Differences between means were assessed with Tukey's method and considered significant at $p<0.05$.

Results: The insect meals largely differed in fat content (meal A: 133 g/kg DM; meal B: 299 g/kg DM). Feed intake varied from 114 (BB) to 123 (AS) g/day and feed conversion efficiency ranged from 1.8 (AS) to 1.9 (SS) g feed per g egg, but did not significantly ($p>0.05$) differ between the treatments. Similarly, the laying performance was not significantly ($p>0.10$) influenced by the use of any of the insect materials in the diets and was approaching 100%. The daily egg mass was lower ($p<0.01$) with BB than with AS (61.0 vs. 68.6 g/d), but both were not significantly different from the control group. With regard to the egg quality traits, there were no effects except for the yolk redness which was higher ($p<0.05$) in BB compared to SS.

Conclusions: The results of the present experiment show that insect meal and fat from different origin can be used instead of soybean cake and oil without substantial impairments of performance of the hens and their egg quality. Ongoing analyses will show whether the insect fat results in a less favourable fatty acid profile in terms of human health.

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* *ETH USYS, Universitätsstrasse 2, 8092 Zurich, Switzerland, maike.heuel@usys.ethz.ch*

12.

Heat treatment of soybean cakes from two soybean varieties differing in protein solubility – effect on broiler performance

Hitzebehandlung von Sojakuchen zweier Sojabohnensorten mit unterschiedlicher Proteinlöslichkeit und deren Einfluss auf die Mastleistung von Broilern

*Hemetsberger F., Kneifel W., Schedle K. – Vienna

Soybean products are an important source of protein in today`s livestock diets. Processing of soybeans prior to feeding is crucial to ensure high digestibility. A thermal treatment is required to minimize the activity of intrinsic anti-nutritional factors like trypsin inhibitors. Yet, if applied excessively, may lead to the deterioration of nutrients. Especially amino acids are prone to degradation by heat and loss of these will affect animal performance. Any heat beyond the amount needed to reduce anti-nutritional factors should be avoided. However, the appropriate treatment may vary as different beans respond variably to heat (1,2). This trial studied the nutritional value of two soybean varieties, processed with two different heat intensities on performance of broilers.

Methods: Soybean varieties were chosen based on their characteristics of decreasing protein solubility in KOH. Soybean variety one showed little decrease in protein solubility during heat treatment. Variety two appeared to be more heat susceptible, its protein solubility declined to a greater extent. For the feeding trial partially defatted soybeans were either treated in an autoclave at 110 °C for 20 minutes (plus 20 minutes for heating-up and cooling-down) to reduce trypsin inhibitors to a desired activity of 3-4 mg/kg or treated at 120 °C for 20 minutes (plus 45 for heating-up and cooling-down) to simulate heat damage. In the 2x2 factorial arrangement the effect of variety and heat treatment was tested in a broiler feeding trial. The feeding trial was carried out for 36 days with 336 one-day-old chickens (Ross 308), distributed equally among 24 pens. Diets included 30% of the experimental soybean cake.

Results: Low heat-treated soybean cakes had residual trypsin inhibitor activities (TIA) of 3.3 and 5.1 mg/g for the applied varieties. High heat-treated cakes had TIA under the limit of detection of 0.5 mg/g in both varieties. Variety one showed increased average daily gain (ADG) and avergage daily feed intake (ADFI) ($p<0.05$), however no differences regarding feed conversion rate (FCR) occurred. An enhanced heat treatment resulted in increased ADG and ADFI, however no differences regarding FCR ($p<0.05$). The observed interaction between variety and heat treatment indicated an increased ADG and ADFI ($p<0.05$) as a result of high heat treatment for variety two but not for variety one.

Conclusions: Sufficient reduction of TIA applying 110 °C for 20 minutes was accomplished only with one of the soybean varieties. The variety known for its high protein solubility decline treated with 110 °C for 20 minutes led to decreased growth performance due to its high TIA of 5.1 mg/g. The applied excessive heat treatment showed no negative effects. As feed conversion rate was identical for all treatments, differences in weight gain appeared to be mainly caused by increased feed intake. It was shown that while a heat treatment is adequate for one soybean variety, it may not be for another one.

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* *University of Natural Resources and Life Sciences Department of Food Science and Technology, Muthgasse 18, 1190 Vienna, Austria, florian.hemetsberger@gmail.com*

13.

Does genetic background of Rainbow trout impact on growth and feed utilization following fishmeal substitution by partly defatted insect meal (*Hermetia illucens*) or microalgae powder (*Arthrospira platensis*)?

Hat die genetische Herkunft bei Regenbogenforellen Auswirkung auf Wachstum und Futterverwertung nach Fischmehlsubstitution durch teilentfettetes Insektenmehl (Hermetia illucens) oder Mikroalgenpulver (Arthrospira platensis)?

*Dietz C., Sünder A., Liebert F. – Göttingen

In recent years the fishmeal content in aquafeed continuously decreased and novel feed ingredients like insects or microalgae are integrated in modern diet formulations. To adapt fish to these dietary changes breeding becomes an important tool. The aim of the current study was to investigate if there are differences in feed utilization and growth performance following substitution of fishmeal (FM) by partly defatted insect meal (*Hermetia illucens*) or microalgae powder (*Arthrospira platensis*) by local and commercial breeds of Rainbow trout.

Methods: A growth trial was conducted with 495 juvenile Rainbow trout (*Oncorhynchus mykiss*; one year old, 111-205g on average) using a control diet based on 20% FM and two experimental diets with 100% substitution of FM by spray-dried Spirulina powder (SP; Crude protein (CP) = 69% of dry matter (DM); diet SP20) or partly defatted *Hermetia* meal (HM; CP = 61% of DM; Crude lipids = 14% of DM; diet HM20). Diets were formulated to be similar both in CP (48% of DM) and digestible energy content. Lys and Met supplementation ensured indispensable amino acid supply as recommended (1). Diets were fed to a commercial breed (Troutlodge Inc; TL) and three local breeds (Hk3, Hk7, Hk8) of trout. Feed utilization and growth response were investigated over 56 days making use of a closed in-door water recirculation system with 36 tanks (320 l/tank; water temperature 15.5 ± 0.5°C; regulated photoperiod 14h light/10h dark) and three replicate tanks per diet (10 fish (TL) or 15 fish (Hk3, Hk7, Hk8) per tank) with fixed feed supply (1.0% of body weight; 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. Growth data (weight gain, WG; specific growth rate, SGR; feed conversion ratio, FCR; protein efficiency ratio, PER) were similar between diets. In contrast, differences in growth performance between breeds under study could be observed whereas Hk7, especially as related to TL, tended to show superior response to the diets under investigation. Hk3 as well as Hk8 were similar to TL regarding WG, SGR, FCR and PER. No significant interactions between breed and diet have been detected. Furthermore, the dietary inclusion of SP yielded a yellowish colour of fillets in all breeds.

Conclusions: Both of the alternative protein sources under study are useful to replace FM in diets for Rainbow trout. Local breeds respond similar or slightly better to test diets than the commercial one. Breed differences in utilizing test diets with alternative protein sources reveal the potential to adapt fish on varying dietary formulations with novel feed ingredients by selective breeding based on local genetic resources. Impact on fillet colour due to SP application needs further consideration as related to consumer acceptance.

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* *Georg-August-Universität DNTW, Tierernährungsphysiologie, Kellnerweg 6, 37077 Göttingen, Germany, cdietz@uni-goettingen.de*

14.

Utilization of duckweed as fish meal replacement in common carp (*Cyprinus carpio*)

Verwertung von Wasserlinsen als Fischmehlersatz durch Karpfen (Cyprinus carpio)

*Stadtlander T., Surber J., Pietsch C., Tschudi F., Sigrist M., Seitz A., Kreuzer M., Leiber F. – Frick / Wädenswil / Zurich

Duckweed, the family Lemnaceae, comprises a group of around 40 different aquatic flowering plant species. They can grow very fast, producing higher biomasses compared to terrestrial plants (up to 79 t DM ha^{-1} a^{-1}, Leng et al. 1995). Furthermore, they are very efficient in uptake of nitrogen and phosphorous and are producing protein of high quality (Stadtlander et al. 2019). Protein contents usually range between 25 to 35% of DM (dry matter) but have been reported to be as high as 45% of DM. Duckweed (*Spirodela polyrhiza*) has been successfully grown on diluted cow slurry and been fed to rainbow trout fry (Stadtlander et al. 2019). Common carp (*Cyprinus carpio*) is among the most important cultured fish species in the world, with a production of 4.13 mio t in 2017, and cyprinids (carp-like fish) in general contribute around 50% of global aquaculture fish production. In this study, we tested two different duckweed (*S. polyrhiza*) meals, one dried (DWD) and one fermented (DWF), in three different concentrations in the diet of carp fry and compared results to a duckweed free control (C).

Methods: The control diet contained 40% fish meal while in the experimental diets 15, 30 and 45% of fish meal protein were replaced by DWD (containing 35% crude protein (CP) and 4.2% crude lipids (CL) resulting in diets DWD15, DWD30 and DWD45) and DWF (containing 27.5% CP, 3.5% CL resulting in diets DWF15, DWF30 and DWF45) protein. Diets were composed to contain 45% CP, 12.5% CL and 17 kJ g^{-1} digestible energy (estimated). The feeds were extruded on a single screw extruder, dried and crumbled. Twenty carp (0.69 g $fish^{-1}$) were put into 10 L aquaria of a recirculation system and the seven different feeds allocated to four replicated aquaria each. Two initial groups of around 60 g fresh mass (~120 fish per group) were killed (150 mg L^{-1} MS-222, buffered with 300 mg L^{-1} NaH_2CO_3) for proximate analysis (baseline data). During six weeks, fish were fed 5% of their body mass per day for 6 days before fasting them for one day, group weighing all fish of each aquarium and adapting feeding rations. At the end of the experimental feeding, all fish were sacrificed and analyzed for proximate composition. Specific growth rate (SGR), relative body mass gain (BMG [%]), feed conversion ratio (FCR), protein productive value (PPV) and lipid productive value (LPV) were evaluated and compared to the control group. Results were compared by ANOVA followed by a Tukey HSD post-hoc test with a significance level of $\alpha = 0.05$.

Results: Feed acceptance for DWD diets was similar as for control diet, whereas the acceptance of DWF diets was inversely correlated to the proportion of fermented duckweed level. After few days, however, even DWF45 was completely eaten by all fish. All carp groups receiving DWD or DWF15 were growing equally well as the control. The BMG [%] ranged between 123 and 128% and SGR between 1.63 and 1.69. The carp receiving DWF45 were significantly inferior in growth to all other groups with BMG of 74% and SGR of 1.13. Growth of carp fed DWF30 were in between, both in terms of SGR and BMG. Feed conversion ratio, PPV and LPV followed the same pattern with C, DWD15, DWD30, DWD45 and DWF15 fed carp being superior to DWF45. Fish fed with DWF30 showed FCR, PPV and LPV ranging between the superior and inferior groups.

Conclusions: The results show clearly that dried duckweed is a suitable source to replace up to 45% fish meal protein without impairing any of the evaluated performance parameters in common carp. Fermented duckweed, however, was competitive to fish meal only in the lowest concentration (15% fishmeal replacement) while performance was reduced with increasing concentrations.

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** FiBL Department of Livestock Sciences, Ackerstrasse 113, 5070 Frick, Switzerland, timo.stadtlander@fibl.org*

15.

Evaluation of the prebiotic potential of dietary cellobiose in healthy adult dogs

Untersuchungen zum präbiotischen Potenzial von Cellobiose bei gesunden adulten Hunden

*Paßlack N., Zentek J. – Berlin

Cellobiose is a disaccharide, consisting of two glucose molecules linked by a β-(1,4′) glycosidic bond (1). Due to the β-(1,4′) glycosidic bond, cellobiose is not degraded by mammalian digestive enzymes, but microbially fermented in the large intestine (2). This might imply a prebiotic potential, as demonstrated for other fermentable carbohydrates (3), but has not yet been evaluated in dogs.

Methods: Ten dogs received a diet without (D0) or with 0.5 g cellobiose/kg body weight (BW)/day (D1) and 1 g cellobiose/kg BW/day (D2) for 3 weeks each. At the end of the feeding periods, samples of feces, urine and blood of the dogs were collected. Feces were analyzed for microbial metabolites, urine and blood for phenol and indole concentrations. Data were compared by the calculation of polynomial contrasts using IBM SPSS Statistics 22.

Results: The addition of cellobiose to the diet markedly increased the fecal L-lactate (in µmol/g; D0: 0.16 ± 0.23; D1: 11.3 ± 9.50; D2: 11.9 ± 15.7; linear contrast: $P = 0.042$) and D-lactate concentrations (in µmol/g; D0: 0.44 ± 1.21; D1: 8.39 ± 7.42; D2: 8.43 ± 9.86; linear contrast: $P = 0.035$) and slightly decreased the fecal actetate (% short-chain fatty acids) concentrations (D0: 53.7 ± 3.31; D1: 52.9 ± 4.90; D2: 49.1 ± 6.22; linear contrast: $P = 0.013$). In addition, phenol (in µg/ml; D0: 2.56 ± 0.32; D1: 2.53 ± 0.13; D2: 2.35 ± 0.21; linear contrast: $P = 0.034$) and 4-ethylphenol (in µg/ml; D0: 0.17 ± 0.06; D1: 0.13 ± 0.05; D2: 0.11 ± 0.02; linear contrast: $P = 0.002$) were decreased in the blood of the dogs, while the urinary phenols and indoles were not affected among the groups.

Conclusions: Due to the marked increase of fecal lactate concentrations, it can be assumed that cellobiose was extensively fermented in the large intestine of the dogs. The reduced phenol and 4-ethylphenol concentrations in the blood might suggest alterations in the intestinal formation or absorption of nitrogen metabolites. Although these results indicate a prebiotic potential of cellobiose, further studies might be necessary to confirm those effects in diseased animals.

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* *Freie Universität Berlin Department of Veterinary Medicine/Institute of Animal Nutrition, Königin-Luise-Str. 49, 14195 Berlin, Germany, nadine.passlack@fu-berlin.de*

16.

Growth performance and carcass characteristics of fattening rabbits fed a complete diet containing shredded acorn

Wachstumsleistung und Schlachtkörperqualität von Mastkaninchen nach Fütterung eines Alleinfutters mit zermahlenen Eicheln als Futterkomponente

*Wolf P., Cappai M. – Rostock / Sassari

In some mediterranean countries, free ranging animals accessing oak forests can directly consume ripe acorns from the ground, or combined into mixed diets when confined, like pigs[1]. In such diets acorns are used as the main energy source for finishers prior to slaughter to produce meat with desirable characteristics. Main nutrient contents in acorns reach between 51–57% of starch and 4–6% of crude fat (dry matter basis) with a high proportion in oleic acid[2]. The question arose as to whether such effects could also be used in rabbit feeding practices. This trial pointed to acquire first data on feeding acorns to fattening rabbits, as to response to dietary tannins and as to carcass characteristics and meat quality.

Methods: This study enrolled 40 weaned fattening rabbits (age: 37 days old; body weight, BW: 703 ± 22.5 g). All animals were grouped according to two dietary treatments (20 rabbits each) divided upon weaning weight and litter. For 39 days a commercial fattening diet (16% crude protein, 14% crude fat) was fed without or with acorns (20% of the diet). Chemical composition of feeds was determined according to proximate analysis and total polyphenols as well as tannin acid equivalent (TAE) were determined[3]. Feed intake was quantified daily, whilst average body weight gain (ADG) once a week. At the end of the experiment (day 76) all rabbits were slaughtered and carcasses were investigated. Chemical composition of polyunsaturated fatty acids (PUFA) in crude fat was determined. In addition, sensory evaluation of meat (5x5 cm of *Musculus quadriceps femoris*) was assessed at roasting using a score (aromatic: - low, + good, ++ very good). Response to dietary tannins was evaluated through parotid gland (*G. mandibularis*) response. Data were analyzed by a two factorial ANOVA using mixed procedure of SAS 9.4 (2012; $p < 0.05$).

Results: The tannic acid equivalents (TAE) of acorn resulted to be 51.6 g/kg DM, providing an amount of 10.2 g TAE/kg DM in the diet with acorns. No significant differences were found in daily feed intake between groups, though ADG were slightly higher in the group fed the acorn combined diet. Due to the acorn content, feed conversion increased from 3.19 ± 0.56 to 3.48 ± 0.31. The carcass weight was almost unchanged and was 1394 ± 89 (control) and 1402 ± 84 g (acorns). The acorns also led to a significant increase in the amount of PUFA in perirenal fat tissue. The addition of acorns increased the proportion of salivary gland from 0.252 ± 0.035 to 0.325 ± 0.028 % of BW. This difference could be statistically verified. In the sensory evaluation 94.7 % and therefore significant more samples of the meat from rabbits fed acorns were assessed to be more aromatically.

Conclusions: Acorns can be used as an energy source in mixed feeds for rabbits (replacement of barley), but may delay the fattening period (fibre content?). The special influence on the carcass quality might improve the opportunities for marketing fattening rabbits.

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* *Chair of Nutrition Physiology and Animal Nutrition, University of Rostock, Justus-von-Liebig-Weg 6b, 18059 Rostock, Germany, petra.wolf@uni-rostock.de*

17.

Nutritive value of haylages for horses with special emphasis on praecaecally digestible crude protein and amino acids as well as on water-soluble carbohydrates

Futterwert von Heulagen für Pferde unter besonderer Berücksichtigung des Gehaltes an praecaecal verdaulichem Rohprotein und praecaecal verdaulichen Aminosäuren sowie wasserlöslichen Kohlenhydraten

*Zeyner A., Meyer R., Steinhöfel O., Greef J.-M., Liebert F. – Halle (Saale) / Göttingen / Köllitsch / Braunschweig

Despite the fact, that haylages are widely used as horse feed in Germany, there are only few data available on energy and nutrient contents, particularly including praecaecally digestible (pcd) crude protein (CP) and amino acids (AA) and water-soluble carbohydrates (WSC). Contents of pcdCP and pcdAA are necessary for diet calculation (1) and WSC to assess possible risks for equines with disturbed insulin sensitivity (2). The aim of the study was to provide respective data from haylages in practical use.

Methods: Totally 50 haylages (different batches) from 33 horse-keeping farms from Lower Saxony were sampled from 15th August to 4th December 2017. The haylages were investigated for dry matter, pH, organic acids, ammonia, Weende nutrients, detergent fibers, pcdCP, pcdAA, individual water-soluble carbohydrates (WSC), 4N HCl-insoluble ash (AIA) and minerals. DM, proximate nutrients and minerals were analyzed according to VDLUFA. Organic acids and alcohols were determined by HPLC and refractive index detection, ammonia by the Conway method. Metabolizable energy (ME), pcdCP and pcdAA were calculated according to (1). Praecaecal digestibility (pcD) of CP was calculated as well. For statistics, regression analysis was used (SPSS25).

Results: The haylage samples contained 740 ± 92.1 g/kg DM. The pH varied at 5.94 ± 0.424. The content of fermentation products was as follows (in g/kg DM): lactic acid 4.9 ± 5.2, acetic acid 2.5 ± 1.5, further volatile fatty acids and alcohols were below the limit of detection. Ammonia-N amounted to 2.3 ± 1.7 % of total N. Furthermore, the haylages contained proximate nutrients as given here (in g/kg DM): crude ash (CA) 85 ± 36, CP 100 ± 25.9, crude fibre 326 ± 41.5, aNDFom 633 ± 73.3, ADFom 363 ± 44.5, ADL 46 ± 13, glucose 13 ± 9.9, fructose 33 ± 20, sucrose 3 ± 7, fructans 32 ± 28, pcdCP 59 ± 2.0, pcdLys 2.27 ± 0.735, pcdMet 0.77 ± 0.214, pcdCys 0.49 ± 0.130, pcdThr 2.30 ± 0.684, Ca 4.76 ± 1.64, P 2.53 ± 0.667, Mg 1.70 ± 0.518, Na 1.00 ± 0.811. Trace elements were (in mg/kg DM): Fe 380 ± 727, Cu 9 ± 3, Zn 31 ± 21, Mn 119 ± 89.9. Eight haylages contained > 100 g CA/kg DM and all 50 on average 20 ± 23 g AIA/kg DM. The pcD CP was 58 ± 7.1 %. The content of pcdCP (y) was positively related to that of CP (x), with a slope of the equation being below 1: $y = - 1.35 + 0.721\ x$ (y and x in g/kg DM; $R^2 = 0.886$; $p < 0.001$). The ME content amounted to 6.6 ± 0.71 MJ/kg DM.

Conclusions: Although the year 2017 did not reveal extreme weather situations, CA, AIA and Fe indicate considerable soil contamination in a high percentage of haylages. The DM content was fairly high and as expected from that, fermentation patterns confirm low lactic acid formation, but also low desmolysis and proteolysis (3). Amounts of 80 g WSC/kg DM as measured here are in the range of what seems to have the potential to elevate postprandial insulin responses in prone animals disproportionately (2).

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* *Martin Luther University Halle-Wittenberg Institute of Agricultural and Nutritional Sciences, Theodor-Lieser-Str. 11, 06120 Halle (Saale), Germany, annette.zeyner@landw.uni-halle.de*

18.

Apparent digestibility of energy and proximate nutrients of ensiled and ensiled + dried partial-crop field peas in sheep

Scheinbare Verdaulichkeit von Energie und Nährstoffen aus siliertem und siliertem + getrocknetem Erbsenschröpfschnitt bei Schafen

*Bachmann M., Kuhnitzsch C., Martens S., Steinhöfel O., Zeyner A. – Halle (Saale) / Köllitsch

Partial-crop field peas may be a valuable source of protein, starch, and fiber in ruminant diets. Fast and safe preservation is commonly achieved by ensiling. Heat treatment might be useful to reduce natively high protein solubility and mitigate elevated protein solubility due to ensiling (1). The objective was to investigate the effect of high-temperature drying of partial-crop pea silage on energy and nutrient digestibility, and contents of metabolizable energy (ME) and net energy lactation (NEL).

Methods: Field peas were cut at 25 cm, which means that ~ 58 % of the plant was harvested. The native partial-crop peas had 327 g DM/kg, 66 g crude ash (CA)/kg DM, 150 g crude protein (CP)/kg DM, 88 g soluble protein/kg DM, 17 g acid ether extract (AEE)/kg DM, 242 g crude fiber (CF)/kg DM, 320 g neutral detergent fiber (aNDFom)/kg DM, 260 g acid detergent fiber (ADFom)/kg DM, and 45 g acid detergent lignin (ADL)/kg DM. Native plants were chopped (Ø 8 mm) before addition of inoculants (*L. plantarum* and *P. acidilactici*; together 4×10^{11} colony forming units per g fresh matter), and ensiled in a silage plastic bag for 146 days. Ensiled peas were dried in a roasting drum (~ 500 °C). The digestion trial was run as difference test according to GfE guidelines (2) using 8 adult wethers on a basal diet (450 g lucerne chaff, 450 g barley, and 100 g soybean meal/d as fed). Test feeds were offered on half of the basal diet. DM and proximate nutrients were analyzed according VDLUFA methods (3). Gross energy (GE) was determined by bomb calorimetry and by regression equation (4). Soluble protein was calculated from protein fractions (5) as sum of non-protein nitrogen (fraction A) and protein, which is soluble in buffer (fraction B1). Organic acids and alcohols were determined by high performance liquid chromatography and refractive index detection. ME and NEL were calculated according to GfE (5). Calculation of NEL based on both, measured GE and calculated GE. Statistical analysis was performed using SAS 9.4 by pooled t-test at $P < 0.05$ significance level.

Results: The sheep' body weight was 68 ± 13 kg and was kept throughout the experiment. The silage had a pH of 4.4, and 83 g lactic acid/kg DM, 28 g acetic acid/kg DM, and less than 1 g/kg DM of other organic acids. Ethanol, 1,2-propanediol, and 1-propanol were 7.4, 6.4, and 2.1 g/kg DM, respectively. The ensiled peas had 317 g DM/kg, 66 g CA/kg DM, 164 g CP/kg DM, 126 g soluble protein/kg DM, 17 g AEE/kg DM, 251 g CF/kg DM, 331 g aNDFom/kg DM, 279 g ADFom/kg DM, 45 g ADL/kg DM, and 18.6 MJ GE/kg DM. Ensiled + dried peas had 908 g DM/kg, 68 g CA/kg DM, 160 g CP/kg DM, 121 g soluble protein/kg DM, 13 g AEE/kg DM, 272 g CF/kg DM, 386 g aNDFom/kg DM, 319 g ADFom/kg DM, 50 g ADL/kg DM, and 18.6 MJ GE/kg DM. Apparent digestibility was 0.75 vs. 0.74 (organic matter), 0.81 vs. 0.81 (CP), 0.49 vs. 0.41 (AEE), 0.53 vs. 0.57 (CF), 0.53 vs. 0.57 (aNDFom), 0.57 vs. 0.61 (ADFom), 0.86 vs. 0.84 (NFE), 0.42 vs. 0.42 (CA), and 0.73 vs. 0.72 (GE) in ensiled vs. ensiled + dried peas ($P > 0.05$). ME was 10.7 and 10.5 in ensiled or ensiled + dried partial-crop peas, respectively ($P > 0.05$); NEL was 6.4 vs. 6.5 and 6.3 vs. 6.3 MJ/kg DM, respectively, based on measured vs. calculated GE ($P > 0.05$).

Conclusions: Protein solubility of partial-crop peas (58 % of CP) increased after ensiling (77 % of CP). High-temperature drying of the silage did not alter protein solubility (76 % of CP), nor did it affect digestibility of energy and nutrients.

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* *Martin Luther University Halle-Wittenberg Institute of Agricultural and Nutritional Sciences, Theodor-Lieser-Straße 11, 06120 Halle (Saale), Germany, martin.bachmann@landw.uni-halle.de*

19.

Suitability of ensiling as preservation method for insect larvae of *Hermetia illucens*

Eignung der Silierung als Konservierungsmethode für Insektenlarven der Hermetia illucens

*Kube K., Resch R., Gierus M. – Vienna / Irdning

Searching for alternative protein sources in animal nutrition, insects gained attention in the last years. Larvae of *Hermetia illucens* are characterized by a crude protein content of about 300 g/kg DM and lipids (~250 g/kg DM) are characterized to contain mostly lauric acid (C12:0). However, sustainability of larvae production largely depends on efficient processing steps. In contrast to previous studies, stating that most of the energy is required for the rearing process (temperature) (1), it has recently been shown that especially drying as part of larvae processing requires the majority (38%) of energy. This, in turn, increases the production costs, while sustainability of larvae production decreases, which subsequently also decreases the competitiveness of larvae in comparison with other protein sources utilized in animal nutrition (2). Nevertheless, the drying step is mandatory since the high water content (70%) of larvae, makes them prone to both enzymatic and non-enzymatic proteolysis as well as microbial deterioration. In consequence, artificial drying is currently the common method to convert fresh to a dry and storable larvae form. Therefore, current research has focused on the impact of different drying techniques on the product (3). However, investigation of alternative conservation methods is needed to improve sustainability of using insect larvae as protein feedstuff. Anaerobic storage using the effects of bacterial lactic acid formation, requires water-soluble carbohydrates as metabolizable substrate for naturally occurring lactic acid bacteria. The resulting low pH ensures a sufficient suppression of harmful microorganisms and thereby preserves the nutritive value. Since the amount of water-soluble carbohydrates in insect larvae is very low, we hypothesized that by the addition of barley meal with or without different silage additives, enough lactic acid production ensures successful conservation of the larvae.

Methods: The present study consisted of three independent experiments aiming to screen for promising larvae ensiling treatments. Treatments in Experiment 1 comprised biological and chemical silage additives, alone or in combination with barley meal so that a wide range (35 in total) of treatments were tested. All treatments were ensiled in triplicate in vacuum bags and pH as well as dry matter loss were used to evaluate ensiling success. In Experiment 2, based on the results of Experiment 1, the most promising treatments (21 in total) were chosen and ensiled again in vacuum bags as well as in glass jars. Moreover, the chosen silages were additionally prepared with larvae in chopped form. Ensiling success was evaluated by determining pH, dry matter loss, crude nutrients and fermentation acids (lactic acid, acetic acid, butyric acid) as well as ammonia-N. Experiment 3 tested the three most promising treatments (40% barley meal, 40% barley meal and sodium nitrite, 40% barley meal and lactic acid bacteria) of Experiment 2. A comprehensive assessment of ensiling quality characteristics was conducted, including determination of crude nutrients, amino acids and fermentation acids. These measurements were additionally conducted for the fresh larvae, dried larvae and untreated barley meal as controls.

Results: Based on all three experiments, the results showed that including barley meal for up to 40% as water-soluble carbohydrate source ensured a sufficient lactic acid fermentation as indicated by a low pH of 4.5, and sufficient amounts of lactic acid (3.0%) along with low amounts of acetic acid (0.56%), butyric acid (0%) and ammonia-N (0.62%). Interestingly, silage quality was not substantially improved by applying silage additive.

Conclusions: The ensiling of *Hermetia illucens* larvae with barley meal seems to be a successful preservation method.

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* *University of Natural Resources and Life Sciences Institute for Animal Nutrition, Lifestock Products and Nutrition Physiology, Muthgasse 11, 1190 Vienna, Austria, kristina.kube@boku.ac.at*

20.

Immuno-detection of oxidative modifications and mass spectrometric identification of processed animal proteins of different origin

Immunodetektion oxidativer Modifikationen und Massenspektrometrische Identifikation verarbeiteter tierischer Proteine unterschiedlicher Herkunft

*Bernevic B.-I., Pieper R., Zagon J. – Berlin

Processed animal protein (PAP) is characterized by a high crude protein content and nutritional value and therefore considered as a potential feed material. PAPs are produced from animal by-products derived from slaughter like e.g. cartilage/bone, muscle fibers, offal or blood. So far, for farmed animals, PAPs are permitted exclusively in aquaculture and only from non-ruminant animals (1). However, exemptions from the strict ban, e.g. for milk protein, still pose an analytical challenge since the official methods for the detection of animal constituents in feed (PCR or light microscopy) are not able to distinguish between illegal ruminant PAP and milk. Mass spectrometry constitutes a solution for this analytical problem (2). However, heat induced modifications may hamper the identification of species specific PAP. In addition, the harsh processing conditions required for PAPs may significantly affect protein quality. In our study we characterized for the first time the oxidative modification profile in heat-treated PAPs. The aim was to identify modified species specific protein targets in PAP to improve mass spectrometrical analysis as well as to assess the impact of processing on protein quality.

Methods: 9 PAPs of different origin (bovine hemoglobin and meal, chicken blood-, meat & bone- and liver meal, pork meal, pork hemoglobin, feather meal and duck meal) were investigated. Proteins were extracted using TCA-acetone and detergent containing buffer and separated by 1- and 2-D-Gel-electrophoresisis. After membrane transfer, carbonylated peptides were derivatized with 2,4-dinitrophenylhydrazine (DNPH) to form hydrazones followed by immunologically detection. Protein spots in 2-D gels were analyzed with Delta 2D analysis programme. Individual spots were picked from the gels, trypsinated and subsequently identified by mass spectrometry using a Q-TOF mass spectrometer (3).

Results: 1-D gel electrophoresis of proteins merely separated when using TCA-acetone during the extraction procedure and produced mostly smears for all PAP samples. In contrast, an alternative approach using detergent containing buffer provided more efficient extraction and protein clean up by showing well resolved bands. All PAP samples revealed +/- visible bands in the1-D carbonyl screening assay. The 2-D gels revealed clearly differentiated spots between different PAPs. Both similar and unique spots among 2-D gels and were used for further LC-MS characterization.

Conclusions: 1-D gel analysis of protein carbonylation represents a sensitive method for the detection of oxidative modifications in praxis samples. For analysis of proteins in more detail 2-D gel analysis can be employed. Mass spectrometry proved to be a powerful tool for protein identification and oxidative structure determination. Besides the identification of non-legislated contamination of feedstuff with ruminant PAP, this may also allow authenticity testing of e.g. pet food and identification of false declarations. Further experiments with PAP mixtures are required to validate the sensitivity of the method. Carbonylation finger prints might finally be also helpful to observe excessive- or deficient heating impacts and consequences for assessment of PAP quality.

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* *German Federal Institute for Risk Assessment, Berlin, Germany, bogdan-ioan.bernevic@bfr.bund.de*

21.

Fibre bound nitrogen proportions of pure sorghum or mixed sorghum-legume silages as affected by temperature and ensiling length

Anteil an fasergebundenem Stickstoff in Silagen aus Sorghum oder Sorghum-Leguminosen-Mischungen in Abhängigkeit von der Siliertemperatur und -dauer

*Aloba T. A., Corea E. E., Dickhoefer U., Castro-Montoya J. M. – Stuttgart / San Salvador

High ambient temperature inherent to the tropics and now more common in temperate regions may affect significantly the nutritional quality of silages, particularly their protein fraction. These temperature effects may depend on the length of ensiling and the original characteristics of the conserved forage. Hence, the aim was to assess the effects of ambient temperature and ensiling length on the proportion of fibre bound nitrogen (N) of sorghum ensiled alone or as mixture with three tropical legumes.

Methods: Sorghum (*Sorghum bicolor*), soybean (*Glycine max*), jack bean (*Cannavalia ensiformis*) and lablab (*Lablab purpureus*) were planted individually. After harvest, the legumes were wilted for 6 h before ensiling. A total of 72 lab-scale silos (400 g fresh matter (FM) each) of sorghum alone or the mixture of sorghum plus each legume (ratio of 60 to 40 FM basis, respectively) were prepared in polythene vacuum bags and conserved for 30, 75 and 180 d. The lab-scale silos were stored either inside a room (indoor) or on the roof of a building under direct sunlight (outdoor) to induce temperatures within the bags. The ambient temperature at the storage locations was monitored using HOBO Pro v2 temperature and humidity loggers at 1 h-interval during the entire period. Samples of the silages at each ensiling time were analysed for neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN). Data were analysed with a general linear model with storage temperature, ensiling length, and their interaction as main effects. Least-squares means were compared using the Tukey test with significance accepted at $P < 0.05$.

Results: The hourly temperature for outdoor and indoor storage ranged from 16-61°C vs 18-35°C,respectively. Storing the silage outdoor showed a higher NDIN and ADIN proportion (g/kg N) compared with storage indoor, reflecting the effects of higher temperatures during in silo conservation. For all silages, ensiling decreased NDIN proportion compared with the original material ($P < 0.01$). For sorghum, NDIN proportion decreased until 30 d and thereafter remained constant until 180 d, whereas for the mixed silages NDIN proportion first decreased at 30 d, increased again until 75 d and then further declined until 180 d ($P < 0.01$). In silages prepared outdoors, ADIN proportion was higher in the ensiled compared with the original material, the difference being greatest at 75 d. When the silages were conserved indoors, ADIN proportion increased until 75 d, but decreased again to levels similar to those of the original forage until 180 d ($P < 0.01$).

Conclusions: Storage temperature has a clear negative effect on fiber-N, highlighting the need to control this factor during ensiling of tropical forages. Ensiling length effect on fiber-N, particularly the proportion of ADIN, appear to be maximized at 75 days of ensiling; but longer ensiling times may decrease both NDIN and ADIN.

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* *University of Hohenheim Department of Animal Nutrition and Rangeland Management in the Tropics and Subtropics, Fruwirthstrasse 31, 70593 Stuttgart, Germany, aloba_temitope@uni-hohenheim.de*

22.

Effect of supplemental rumen-protected methionine on growth and slaughter performance of Fleckvieh bulls for fattening at crude protein deficit

Einfluss einer Zulage von pansengeschütztem Methionin auf Mast- und Schlachtleistung von Fleckviehbullen im Proteinmangel

*Inhuber V., Windisch W., Spiekers H., Ettle T. – Freising / Grub

Improving protein efficiency is a major aim in feeding ruminants. The general strategy comprises reduction in dietary crude protein (CP) at simultaneously improving amino acid quality, e.g. through supplementation of rumen-protected, essential amino acids (1,2). While Lysine and Methionine are considered to be the first limiting amino acids in lactating dairy cows (1,2), the respective relevance to bulls for fattening is still widely unknown. For this reason our project focused on the effect of supplemental rumen-protected methionine (RPM) in a CP deficient diet on growth and slaughter performance of fattening German Fleckvieh bulls.

Methods: The study involved three diets, a control diet (CON) with sufficient CP (13.5% of DM), a protein-deficient diet containing 8.5% CP of DM (NEG) and a NEG diet supplemented with RPM (NEGM). All diets were composed at an isoenergetic level (11.6 MJ ME/kg DM) and both NEG and NEGM were supplemented with rumen-protected Lysine (0.18% Lys in DM) in order to hold the Lysine content constant to the control diet and to further exclude Lysine from acting as first limiting amino acid. RPM was added to the NEGM diet with 0.12% in DM. A total of 69 young bulls of 238 days age and 367 kg of body weight were allotted to the three diets (n=23 per group) and were fed ad libitum for up to 342 days on average. Starting 57 days after the onset of the study, subgroups of bulls were slaughtered weekly. Individual feed intake was automatically recorded daily while the live weight was recorded every four weeks and at the end of the experiment.Statistical evaluation included ANOVA, multiple comparisons of means (SNK test) as well as linear contrasts (SAS 9.4).

Results: Reduction of CP supply depressed DMI (9.43 kg/day of CON vs. 8.38 kg/day as average of both NEG and NEGM, $p<0.0001$), and DWG (1580 vs. 1228 g/day, $p<0.0001$), respectively. Within the protein-deficient diets, addition of rumen-protected methionine did not alter DMI or DWG to a statistically significant extent (8.49 vs. 8.27 kg/d, $p=0.44$; 1256 vs. 1199 g/day, $p=0.45$). Dressing percentage (56.7 vs. 55.6 %; $p< 0.001$) and carcass weight (298 vs. 273 kg, $p<0.01$) was higher in CON compared to NEG/NEGM groups but there was no difference among CP reduced groups. Carcass classification was not influenced by treatment. Fat classification indicated higher fatness in CON compared to NEG/NEGM groups (2.35 vs 2.09 points, $p< 0.01$), but this was not reflected by amount of kidney fat or intramuscular fat content. Parameters of carcass fatness were not different between CP deficient groups. Meat pH value, meat color, cooking and storing losses of meat were not influenced by treatment. Higher shear force value in CON compared to NEG/NEGM groups (53.1 vs. 45.9 N, $p<0.05$) indicated more tenderness of meat due to CP reduced feeding. There was no difference in shear force among CP reduced groups.

Conclusions: Depressed DMI, DWG and carcass weight demonstrated efficacy of protein restriction in feeding group NEG. Lack of effect of supplemental rumen-protected methionine suggests that methionine was either not the first limiting amino acid under the condition of elevated Lysine supply or the next limiting essential amino acid was very close to methionine.

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* *Technical University of Munich Chair of Animal Nutrition, Liesel-Beckmann-Straße 2, 85354 Freising, Germany, inhuber@wzw.tum.de*

23.

Effect of rumen-protected methionine and lysine supplementation on the developmental competence of bovine cumulus-oocyte-complexes collected from dairy cows via OPU

Einfluss einer pansengeschützten Methionin- und Lysin-Supplementation auf die Entwicklungskompetenz boviner Kumulus-Oozyten-Komplexe (gewonnen mittels OPU von Milchkühen)

Ritz M., Gonzalez A., Fries A.-S., Scheu T., Blad-Stahl N., Kotarski F., Schuler G., Koch C., *Wrenzycki C. – Giessen / Münchweiler an der Alsenz

Important nutritional elements in dairy cow reproduction are amino acids (AA). Supplementation of rumen-protected AA (RPAA) has proven to be an effective tool to supply limiting AA in dairy diets. Methionine and lysine are the two most limiting AA for lactating dairy cows. There is growing evidence that oocyte maturation, fertilization, and preimplantation embryonic development are particularly sensitive periods to changes in maternal nutrition. Recently it has been shown that methionine supplementation seems to impact preimplantation embryos collected from superovulated cows enhancing their developmental competence as there is strong evidence that endogenous lipid reserves serve as an energy substrate (1). Moreover, higher concentrations of methionine were determined in the follicular fluid of the first dominant follicle post-partum in cows supplemented with rumen-protected methionine and rumen-protected choline between 21 days before calving to 30 days post-partum and it was assumed that higher methionine concentrations in the follicular fluid could affect oocyte quality (2). There is no information available so far regarding the effect of a combined methionine and lysine supplementation (each rumen-protected) on oocyte quality. Therefore, the objective of this study was to evaluate the effect of a combined methionine and lysine supplementation during early to mid-lactationon the developmental competence of oocytes collected from lactating dairy cows (days 0 – 100 p. p.).

Methods: Thirty pregnant multiparous German Holstein dairy cows were grouped 3 weeks before their expected calving date receiving identical diets. After calving, they were randomly allocated to two groups fed a total mixed ration supplemented with (N=14 cows; RPAA) or without (N=16 cows; CON) encapsulated lysine and methionine adsorbed onto a silicon dioxide carrier (15 g and 10 g, respectively). Starting from 45 days p. p. onwards, animals from both groups were submitted to an ovum pick-up (OPU) session once a week for at least 8 weeks. Collected cumulus-oocyte-complexes (COC) were subjected to a standard in vitro production (IVP) protocol (3) including in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC). Cleavage and developmental rates up to the morula/blastocyst stage were recorded on days 3, 7 and 8 (D3, D7, D8). All statistical analyses were performed with SigmaStat (version 3.5, Systat Software GmbH, Erkrath, Germany). Data were analyzed using t-test. To be statistically significant a p-value ≤ 0.05 was considered.

Results: In total, 1211 follicles have been aspirated from RPAA animals compared to 1413 from CON animals from which 742 and 885 COC were collected, respecitvely. The calculated recovery rate based on the number of aspirated follicles and collected COC was similar for both groups (61.3±29.4% vs 62.6±33.5%; P>0.05). Cleavage and developmental rates based on 240 (RPAA group) and 299 (CON group) COC also showed similar results (RPAA: D3: 84.1±5.9% [202/240], D7: 18.3±4.4% [44/240], D8: 18.8±4.7% [45/240]; CON: D3: 81.9±8.6% [245/299], D7: 15.4±8.9% [46/299], D8: 16.7±8.4% [50/299]; P>0.05).

Conclusions: Supplementation of RPAA (methionine and lysine) had no beneficial effect on the developmental competence of COC obtained from these animals compared to those collected from cows fed the diet without RPAA supplementation.

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* *Justus-Liebig-University Giessen Chair for Molecular Reproductive Medicine, Clinic for Veterinary Obstetrics, Gynecology and Andrology, Faculty of Veterinary Medicine, Frankfurter Str. 106, 35392 Giessen, Germany, christine.wrenzycki@vetmed.uni-giessen.de*

24.

Nitrogen intake, metabolism and excretions of dairy cows with divergent milk urea concentrations

Stickstoffaufnahme, -stoffwechsel und -exkretionen von Milchkühen mit unterschiedlichem Milchharnstoffgehalt

*Müller C. B. M., Görs S., Tuchscherer A., Reinsch N., Wimmers K., Kuhla B. – Dummerstorf

The milk urea nitrogen (MUN) concentration is positively correlated with daily urinary urea nitrogen (UUN) excretions from dairy cows with the dietary crude protein level as main factor positively influencing MUN and UUN (Burgos 2007). Despite feeding the same ration and producing comparable milk yields, inter-individual differences in milk urea concentration can be found within herd. Therefore, the objective of the present project was to elucidate nitrogen intake, metabolism and excretions of dairy cows with divergent milk urea content fed different planes of crude protein.

Methods: Twenty German Holstein cows with high (HMU: 277 ± 9 mg/L; n=10) and low (LMU: 189 ± 12 mg/L; n=10) milk urea concentration during 5 consecutive monthly test recordings were purchased from a commercial farm. Cows were at the end of 2nd to 4th lactation and fed two isocaloric diets (10.2 ± 0.1 MJ metabolizable energy/kg dry matter) with a low (LP; 13.7 ± 0.3%) and a higher (HP; 15.8 ± 0.2%) crude protein level for 4 weeks in a crossover design. In the last week on each diet, animals were equipped with a urinal and fed 95% of their ad libitum intake. Feed intake, feces and urine excretions and milk production were measured daily. Subsamples were taken to analyze nitrogen metabolites and total nitrogen to calculate daily excretions, nitrogen balance and utilization efficiency. Furthermore, a ^{13}C-urea bolus was administered intravenously followed by withdrawal of a series of blood samples to assess the urea pool, urea turnover rate and microbial urea oxidation (Wolfe et al. 2005). Based on plasma and urinary creatinine concentrations the renal urea clearance rate was calculated as described previously (Spek et al., 2013). Statistical analysis was carried out using the SAS software for Windows, version 9.4 (Copyright, SAS Institute Inc., Cary, NC, USA). Data was analyzed by repeated measurement ANOVA including the factors milk urea concentration, diet and their interaction and Pearson correlation analysis. Statistical significance level was considered at $P < 0.05$.

Results: Milk yield and nitrogen intake did not differ between HMU and LMU cows. HMU cows showed higher milk urea concentrations and daily excretion ($P < 0.01$) and a greater urea pool ($P < 0.05$) on the HP diet, whereas they had higher milk uric acid concentrations on the LP diet compared to LMU cows ($P < 0.05$). While there was a linear correlation between urine urea and milk urea concentrations when all cows and diets were included ($r = 0.623$; $P < 0.01$), there was no group difference in urine urea excretion. However, urea concentrations in urine ($P < 0.01$), milk ($P < 0.01$) and plasma ($P < 0.05$) as well as the renal urea clearance rate ($P < 0.05$) increased, whereas milk uric acid concentration ($P < 0.05$), nitrogen utilization efficiency and microbial urea oxidation ($P < 0.01$) decreased with increasing dietary crude protein level. In addition, HMU cows had higher milk creatinine excretions on both diets ($P < 0.01$), whereas LMU cows showed higher urinary creatine concentrations on the HP diet ($P < 0.01$) and a higher renal creatinine clearance rate on the LP diet ($P < 0.05$).

Conclusions: Differences in milk urea concentrations between cow groups were not paralleled by urine urea excretions, but associated with differences in kidney function and muscle metabolism.

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* *Leibniz-Institute for Farm Animal Biology Institute of Nutritional Physiology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany, mueller.carolin@fbn-dummerstorf.de*

25.

Optimal methionine plus cysteine requirement in broiler starter diets supplemented with L-methionine

Optimaler Bedarf an Methionin und Cytein im Broiler-Starterfutter bei Zugabe von L-Methionin

Millecam J., Khan D. R., Dedeurwaerder A., *Saremi B. – Izegem / Schwalbach

Methionine (Met) is the first limiting amino acid in broilers and together with cysteine (Cys) are two crucial amino acids to be met correctly. Methionine is supplemented in feed in powder form i.e. DL-Met and L-Met, or in liquid form (DL-Hydroxy analogue). In the absence of L-Met, the sulphur amino acid (SAA) requirement of broilers are defined in DL-Met supplemented diets.

Methods: The objective of this trial was to investigate the optimal SAA requirement of broilers in the starter phase (first 10 days of life) in diets supplemented with L-Met. A basal diet containing 1.28% standardized ileal digestible (SID) Lys and 0.60% SID Met plus Cys (50% Met and 50% Cys; deficient in SAA) was created. L-Met was added to the basal diet to reach 0.65, 0.70, 0.75, 0.85, 0.95 and 1.05% SID Met plus Cys (6 replicates per treatment and 15 animals per replicate). The body weight (BW), daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) were measured. Additionally, feed samples of the test diets were analysed to determine major nutrients including amino acids which appeared to be at or close to formulated values.

Results: After 10 days on the test diets, significant differences ($P < 0.05$, analysed via linear regression) were observed in all measured parameters. The optimal Met plus Cys requirements were determined using linear broken line and exponential asymptotic models. Model fit was evaluated based on the R^2 of individual observations and graphical interpretation. The linear broken line model showed the best fit which was demonstrated with a higher R^2 value of 0.82, 0.81 and 0.41 for BW, DWG and DFI, respectively. The models based on FCR showed a poor fit. Based on the fitted models, the optimal SID Met plus Cys level was found to be 0.69-0.70%. This new value is 20% lower compared to the recommendations of NRC and Ross 308 (0.90% and 0.94% SID Met plus Cys, respectively). These recommendations are defined in diets based on DL-Met. Performance of broilers based on Ross 308 at day 10 of age is 321g BW.

Conclusions: In the present study, chickens at the requirements for the maximum performance needed 20% lower SID Met plus Cys while performing similarly (326 g BW). Due to the scarcity of data, no proper comparison with DL-Met supplemented diets could be made. The lower Met plus Cys requirements might be due to the higher bioavailability of L-Met compared to DL-Met and other complex biological processes. Moreover, nowadays broilers are receiving lower crude protein diets, no antibiotic growth promotors, and have no access to animal protein sources. These huge changes in broilers feeding might also be associated with the lower Met plus Cys requirements in trials based on L-Met as compared to the conventional requirements which has been based on DL-Met.

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* *CJ Europe GmbH Technical Center, Ober der Röth 4, 65824 Schwalbach, Germany, b.saremi@cj.net*

26.

Effects of supplemented non-essential amino acids in diets with very low crude protein concentration on growth and nitrogen utilisation efficiency of broiler chickens

Effekte von Ergänzungen nichtessentieller Aminosäuren in Rationen mit sehr niedriger Rohproteinkonzentration auf das Wachstum und die Stickstoffverwertung von Broilern

Hofmann P., Siegert W., Naranjo V., *Rodehutscord M. – Stuttgart / Hanau

The strong reduction of dietary crude protein (CP) concentration can cause a reduced growth of broiler chickens even when the supply of essential amino acids (AA) and glycine equivalent (Gly_{equi}) is adequate. It has been suggested that non-essential amino acids (neAA) can become growth-limiting in such diets (1). Non-protein nitrogen (NPN) might be used for synthesis of limiting neAA in broiler chickens. This study investigated effects of supplementing single neAA and NPN in very low CP diets for broiler chickens.

Methods: Male Ross 308 hatchlings were provided a commercial starter diet until day 7 of the experiment. Nine birds each were then allocated to 81 metabolism units. Each of the 9 diets was provided to 9 replicated metabolism units until day 21. Two maize-soybean meal-based diets with different neAA concentrations and CP levels of 180 g/kg (CP180) and 160 g/kg (CP160) were mixed. Dietary Gly_{equi} was set at 15 g/kg in all diets based on a preliminary study (1). Another six CP160-based diets were supplemented with either l-alanine (CP160+Ala), l-proline (CP160+Pro), l-aspartic acid (CP160+Asp), a mix of l-aspartic acid and l-asparagine·H_2O (CP160+Asp+Asn), l-glutamic acid (CP160+Glu), or a mix of l-glutamic acid and l-glutamine (CP160+Glu+Gln). Supplements were made to achieve the respective neAA concentration of CP180. In another diet, ammonium chloride (NH_4Cl) was added as the NPN source to CP160 to achieve the CP concentration of CP180. Concentrations of all other nutrients, including essential AA, were calculated to meet the supply recommendations (2). Growth and feed intake were determined on a metabolism unit basis. Total excreta were collected in 12-h intervals from day 18 to 21 and nitrogen utilisation efficiency (NUE) was calculated. Results were statistically analysed by one-way ANOVA using SAS 9.4. The statistical significance was set at $P<0.050$.

Results: The highest average daily gain (ADG) of 59.1 g and gain:feed ratio (G:F) of 0.78 g/g were observed for CP180 ($P\leq0.010$). Feeding CP160 significantly decreased ADG and G:F to 54.2 g and 0.75 g/g compared to CP180 ($P<0.001$). Addition of Asp+Asn (57.1 g), Glu (56.5 g), and Glu+Gln (56.2 g) significantly increased ADG compared to CP160 ($P\leq0.011$). Addition of Asp (0.76 g/g), Asp+Asn (0.77 g/g), Glu (0.76 g/g), and Glu+Gln (0.77 g/g) significantly increased G:F compared to CP160 ($P\leq0.033$). The ADG in CP160+Asp+Asn was significantly higher than in CP160+Asp (by 1.7 g) ($P=0.025$). The G:F was not different between CP160+Ala (0.75 g/g), CP160+Pro (0.75 g/g), and CP160+Asp (0.76 g/g) ($P\geq0.237$). The NUE was highest in CP160 (79.1 %) and significantly lower in CP180 (73.0 %) ($P<0.001$). No significant difference was found in CP160+Ala (78.6 %), CP160+Pro (78.4 %), and CP160+Glu (78.4 %) compared to CP160 ($P\geq0.092$). Addition of Asp, Asp+Asn, and Glu+Gln to CP160 significantly decreased NUE by 1.7 to 2.7 percentage points ($P<0.001$). The NUE was significantly higher in CP160+Asp+Asn (77.4 %) than in CP160+Asp (76.4 %) ($P=0.024$). Overall, significantly lowest ADG (36.2 g), G:F (0.71 g/g), and NUE (71.5 %) was found in CP160+NH_4Cl ($P\leq0.002$).

Conclusions: These results demonstrate that individual supplementation of Asp+Asn, Glu, and Glu+Gln partly compensates for growth-reducing effects of very low CP diets. Increase in growth upon addition of these neAA was the same. Adding Glu is more efficient than Asp+Asn and Glu+Gln to increase NUE. NH_4Cl as a NPN source was found unsuitable to increase growth and NUE.

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* *Universität Hohenheim, Institut für Nutztierwissenschaften, Stuttgart, Germany, markus.rodehutscord@uni-hohenheim.de*

27.

Encephalopathy and blindness in turkeys caused by a methionine oversupply

Encephalopathie und Blindheit bei Puten infolge einer Methioninüberversorgung

*Wolf P., Bahrenberg P., Mandischer C., Lehmbecker A. – Rostock / Hamminkeln / Hanover

A supplementation of the first-limiting amino acid methionine to poultry diets is a common practise in animal husbandry with the ambition to increase growth performance. However, negative consequences (for example a reduced feed intake) due to an oversupply are also described (1, 2).

Case report: The present case occurred on a farm with in total 29,500 turkeys. Animals at an age of five to six weeks showed a reduced feed intake after offering a new complete diet (change from P2 to P3) within a few days, which resulted in lower body weight gains and a higher feed conversion ratio (FCR). Moreover, in around 7 % of these turkeys coordination and movement disorders could be observed. In addition, a lot of them showed blindness of different severity. In the following days the mortality rate increased. Microbiological investigations of died birds did not revealed any evidence or information indicating an infection. In spite of blindness no histological changes of the eyes could be detected. In a pathohistological examination a cerebral encephalopathy was diagnosed.

Since the exchange of the feed batch led to a partial regeneration of the affected animals, the new complete diet (P3) was under suspicion to be the cause for this happening.

Methods: A representative aliquot was divided from the suspect complete diet. Proximate analysis was carried out according to VDLUFA methods. Minerals and heavy metals were analysed after microwave ashing by atomic absorption spectrometry and colorimetry, respectively. Methanol, vitamine A, coccidiostats, pesticides and amino acids as well were determined by HPLC. Moreover, the microbial status of the diet was proven using conventional cultural methods.

Results: The analysed feedstuff contained a crude protein level, which was slightly above the labelled content (analysed: 258 g/kg, declared: 230 g/kg), but deviation was within the tolerance range given by Legislative Decree No 767/2009. In spite of it the analysed methionine level deviates significantly from the declared content (5.60 g/kg). In a triple repetition levels of 13.2/16.7 and 21.5 g/kg were found, that can be explained by an addition of a finely granulated methionine supplement. Moreover, the sulphur content of the diet amounted 6.27 g/kg and was higher than usual (2.0 – 2.6 g/kg). All other results could be classified as being within a normal range.

Discussion: Under physiological conditions methionine is degraded with ATP participation to S-adenosyl-methionine and subsequently to homocysteine. In cases of an oversupply the degradation is carried out by a transamination and by chemical intermediates mercaptans are created. Methanethiol belongs to the group of mercaptans and is usually metabolized in liver tissue. In cases of an oversupply the reduction in the liver tissue decreases and mercaptans and a hyperammonaemia as well cause encephalopathies and blindness (1, 2) as it was observed in this case report.

Conclusions: In cases of clinical signs that occur with a feed change, focus should also be given to the new batch. Regarding feed additives it should be considered that not only an undersupply but also an oversupply can cause severe health disorders as it was observed in this case concerning methionine.

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* *Chair of Nutrition Physiology and Animal Nutrition, University of Rostock, Justus-von-Liebig-Weg 6b, 18059 Rostock, Germany, petra.wolf@uni-rostock.de*

28.

Effect of rumen-protected lysine on zootechnical performance of young fattening Fleckvieh bulls

Einfluss von pansengeschütztem Lysin auf die zootechnische Leistung von jungen Mastbullen der Rasse Fleckvieh

*Riepl F., Inhuber V., Brugger D., Kuenz S., Windisch W., Spiekers H., Ettle T. – Grub / Freising

Reducing dietary crude protein (CP) concentration and concurrent improvement of protein quality, according to specific essential amino acid demand is regarded as an appropriate practice to reduce the environmental impact of cattle farming. Supplementation of rumen-protected essential amino acids is a promising method to increase protein efficiency and reduce nitrogen losses. It is assumed that methionine and lysine are the most limiting amino acids in dairy cows. Nevertheless, the amount of available data concerning fattening bulls is quite low. A recent project investigated the effect of rumen-protected methionine on zootechnical performance of fattening bulls (1). The present study focused on the effect of supplemental rumen-protected lysine on daily weight gain (DWG) and dry matter intake (DMI) of German Fleckvieh bulls during the early stages of fattening.

Methods: This experiment comprised 67 German Fleckvieh bulls with an average age of 184 days and 223 kg live weight. The bulls were randomly allocated to three different diets. A diet sufficient in crude protein (14% CP of DM) served as control (CON; n=22). The second diet (NEG; n=22) was reduced in CP (11% CP of DM), while the third diet (LYS; n = 23) was equivalent to NEG but supplemented with rumen-protected Lys (0.42% Lys in DM). All diets were formulated on an isoenergetic level (11.64 MJ ME/ kg DM) and contained rumen-protected methionine (0.11 % of DM) in order to keep the methionine level constant and to avoid potential limitations by this essential amino acid. The animals were fed ad libitum up to 119 days. Starting with experimental day 63, subgroups of bulls were slaughtered at weekly intervals until day 119. Data collection included live weight at experimental start (LWstart) and end (LWend), total weight gain (TWG) and daily weight gain (DWG). Statistical analyses involved analysis of covariance (group, days until slaughter (DUS), LWstart, group*DUS and orthogonal contrasts (CON vs. NEG+LYS, NEG vs. LYS) (SAS 9.4).

Results: Protein restriction depressed LWend, TWG and DWG significantly ($p<0.0001$) by 8.7%, 20.7%, and 22.2% on average compared to control. Within restricted animals (group LYS), addition of lysine improved LWend, TWG and DWG in tendency ($0.09 < p < 0.07$) by 3.5%, 9.6%, and 8.3%, respectively, relative to group NEG.

Conclusions: The feeding model induced an alimentary CP deficiency as indicated by the significant differences between animals fed 14% vs. 11% CP. This established the methodological basis to investigate potentially limiting amino acids. Interestingly, CP deficient animals which were fed rumen protected lysine tended towards a higher zootechnical performance than NEG animals. Given the simultaneous supplementation of rumen protected Methionine to all groups; this indicates that the amount of lysine at the duodenum was one, but presumably not the only limiting factor. Future studies must investigate whether the addition of further rumen protected amino acids is able to compensate for a limiting supply with dietary nitrogen in the feeding of young bulls.

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* *Bayerische Landesanstalt für Landwirtschaft Institut für Tierernährung und Futterwirtschaft, Prof.-Dürrwaechter-Platz 3, 85586 Grub, Germany, florian.riepl@lfl.bayern.de*

29.

Milk and blood urea nitrogen contents in Brown Swiss and Holstein dairy cows under different feeding conditions

Harnstoffgehalte in Milch und Blut bei Brown Swiss und Holstein Milchkühen bei unterschiedlichen Fütterungsbedingungen

*Kessler E. C., Bruckmaier R. M., Gross J. J. – Bern

The agricultural sector is expected to contribute to the reduction of nitrogen (N) emissions since farm animals excrete considerable N amounts via feces and urine. In order to reduce N emissions by dairy cattle, methods predicting accurately individual N emissions are of great interest. In this context, milk urea content is currently used in models to estimate the N excretion of dairy cows because of the close relationships between milk urea nitrogen (MUN) and urinary nitrogen. Milk urea nitrogen content is closely related to plasma urea nitrogen (PUN) concentration, and reflects the balance of dietary crude protein and energy supply for rumen fermentation. The present study investigated if the higher MUN content in Brown Swiss (BSW) compared with Holstein (HOL) cows is related to milk production and feeding conditions. In addition, the relationships between PUN and MUN assessed either by an enzymatic method or by infrared spectroscopy were analyzed.

Methods: In experiment 1, milk and blood samples (1,112 samples each) were collected in parallel bi-weekly from d 5 until d 150 of lactation from 72 BSW and 69 HOL cows housed at four different farms (each two farms with BSW and HOL cows) with diverse feeding regimens. Parities were 3.3 ± 0.3 (mean ± SEM; range from 1 to 12) for BSW and 2.4 ± 0.2 for HOL (range from 1 to 9). Blood samples for determination of PUN concentrations were obtained from the coccygeal vein with evacuated EDTA-coated tubes. Milk urea concentration was determined by an enzymatic assay and by FTIR spectroscopy to show the correlation between the two methods. Plasma urea concentrations were measured with an enzymatic assay. Experiment 2 consisted of test-day records of three consecutive official milk recordings from 86 BSW and 200 HOL cows kept on the same farm under identical feeding and management conditions. Statistical analysis was performed with SAS (version 9.4). All data were checked for normal distribution with the UNIVARIATE procedure. Concentrations of MUN measured in all milk samples of the first experiment (n = 1,112) by FTIR (MUN_{IR}) and by the enzymatic method (MUN_{ENZ}) were regressed on PUN concentrations determined in the concomitantly obtained blood samples using the procedure REG. In addition, a Bland–Altman analysis was conducted that revealed the agreement between both PUN and MUN_{IR}, and between PUN and MUN_{ENZ}, i.e., to which extent MUN_{IR} and MUN_{ENZ} represent PUN concentration. For experiment 1, effects of breed, time relative to parturition, and the breed × time interaction as fixed effects and the individual cow as repeated subject were assessed with a mixed model. Farm had no effect on MUN and was removed from the statistical model. For the second experiment, the MIXED procedure was used to evaluate effects of breed on MUN content, milk yield, yield of ECM, contents of milk fat, protein, lactose, and SCC with breed, time relative to parturition, and the breed × time interaction as fixed effects and the individual cow as repeated subject. Tukey's t-test results were considered significant at p-values < 0.05.

Results: Both MUN_{IR} and MUN_{ENZ} were highly correlated with PUN (r = 0.93 and 0.89, respectively). Concentrations of MUN and PUN were higher in BSW compared with HOL independently of lactational stage, parity and feeding regimen ($p < 0.0001$). Protein and fat content were higher in BSW than in HOL. Primiparous cows had lower milk yield and ECM ($p < 0.001$ for BSW, $p < 0.0001$ for HOL) than multiparous in both breeds. Multiparous BSW had a similar milk production than primiparous HOL ($p = 0.13$).

Conclusions: BSW cows have higher MUN than HOL under different as well as identical feeding conditions. The elevated MUN in BSW cows is not related to milk production, but rather seems to be an inherent genetic characteristic. Potential differences in the rumino-hepatic N circulation between breeds need to be elucidated.

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* *University of Bern Veterinary Physiology, Vetsuisse Faculty, Bremgartenstrasse 109a, 3012 Bern, Switzerland, evelyne.kessler@vetsuisse.unibe.ch*

30.

Nitrogen balance of dairy cows fed different silage-based diets with and without *Acacia mearnsii* tannins

Stickstoffbilanz von Milchkühen bei Verfütterung verschiedener Silagerationen mit und ohne Acacia mearnsii-Tanninen

*Lazzari G., Münger A., Eggerschwiler L., Zähner M., Kreuzer M., Schrade S., Dohme-Meier F. – Posieux / Zurich / Tänikon

Feeding high proportions of forage to ruminants reduces the competition for food and feed that can also be consumed by humans and monogastric animals. Conserved forages derived from herbage are often high in rumen-degradable crude protein (CP) contents. Therefore, diets should be designed to prevent excessive ruminal protein degradation and to improve N efficiency.Supplementation of tannins extracted from the bark of *Acacia mearnsii* has been shown to reduce urinary N losses (1). The objective of the study was to investigate the effect of tannins supplemented tothree silage-based diets varying in forage type on the N balance of dairy cows.

Methods: In a 6 × 6 Latin Square arrangement, six multiparous Holstein cows (milk yield: 36.6 ± 3.9 kg/d; 70 ± 13 d in milk) were blocked by milk yield and assigned randomly to six treatments. Each experimental period lasted 21 d including a 14-d adaptation and a 7-d data collection period where feed intake, milk yield and milk composition were recorded daily and excreta were completely collected. Cows received one of six total mixed rations at *ad libitum* access which contained (on dry matter (DM) basis) 79% silage and 21% concentrate. The silages used were either ryegrass-rich (GR) or red clover-rich (LR) or composed of tanniferous sainfoin (SF). Concentrates were added to the rations in a way that at least the minimal requirements of the cows for energy, CP and minerals were covered. In addition, rations were supplemented at 2.1% of the total diet either with an extract rich in tannins prepared from the bark of *A. mearnsii* (Baeck GmbH & Co. KG., Norderstedt, Germany) or with straw meal. Data were analyzed by linear mixed models with type of forage, tannin supplementation and their interaction (if significant) as fixed factors.

Results: Dry matter intake was similar for cows fed LR (26.2 kg/d) and SF (24.8 kg/d), and was lower ($P < 0.05$) for cows fed GR (22.6 kg/d). Tannin supplementation tended ($P = 0.07$) to reduce intake. Cows receiving LR had a higher ($P < 0.05$) milk yield (30.4 kg/d) than cows fed GR (24.7 kg/d), and cows fed SF (27.3 kg/d) were intermediate. Milk protein content was higher with LR (3.39%) compared to SF (3.29%, $P < 0.05$), whereas GR did not differ ($P > 0.05$) from the two other forage types. Tannin supplementation caused a decrease in milk yield and in contents of fat and protein ($P < 0.01$). Due to the different forage types, the dietary CP contents varied considerably, being highest for LR (201 g/kg DM), followed by SF (169 g/kg DM) and GR (129 g/kg DM). Consequently, N intake was highest ($P < 0.05$) when cows were fed LR (829 g/d), followed by SF (660 g/d) and GR (457 g/d). The N excreted with milk and the body N retention was highest ($P < 0.05$) when cows fed LR followed by SF and GR, but the ranking was different when utilization of dietary N for milk N was calculated. The N was most efficiently used ($P < 0.05$) when cows fed GR (28.6%) followed by SF (21.5%) and LR (19.9%). Feeding of tannins led to a decrease ($P<0.01$) in N excreted with milk. Fecal N excretion was similar with SF and LR and was lower ($P < 0.05$) with GR. Cows fed LR had the highest ($P < 0.05$) urinary N excretion and total N excretion with excreta followed by SF and GR. Tannin supplementation caused an increase ($P < 0.01$) in fecal N excretion and a decrease ($P < 0.001$) in urinary N excretion resulting in a similar ($P = 0.42$) total N excretion with excreta among diets. The proportion of urinary N (% of N intake) was highest ($P < 0.05$) with LR (20%) followed by SF (13%) and GR (10%). Urinary urea N excretion ($P < 0.05$) and the proportion of urea N in total urinary N were highest ($P < 0.05$) when cows fed LR, followed by SF and GR. Tannin supplementation tended ($P = 0.08$) to decrease urinary urea N excretion.

Conclusions: The forage with the highest CP content increased milk yield probably due to a concomitantly higher energy supply, but also resulted in very high urinary N and urinary urea N excretion, suggesting an inefficient ruminal N utilization. The known shift in the N excretory pattern from urinary to fecal excretion with tannin supplementation was apparent regardless of the dietary CP content.

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* *Agroscope Ruminant Research Unit, Posieux, Switzerland, giovanni.lazzari@agroscope.admin.ch*

31.

Effects of supplementing arginine, glutamine or both to a lactation feed on nursing performance in a commercial sow herd

Effekte einer Supplementierung von Arginin, Glutamin oder beidem auf die Aufzuchtleistung von Sauen in einem Produktionsbestand – Ein generationsübergreifender Versuch unter praktischen Bedingungen

*Wessels A. G., Zentek J. – Berlin

Within the last decade, the number of weaned piglets per sow and year has increased by approximately 30%. This development coincides with enhanced piglet mortality and impaired health of the sows. With litter sizes, also within-litter heterogeneity increases with antagonistic effects on piglet vitality. Underweight piglets lag behind their heavier littermates constantly until the end of the production cycle. A constraint is the limiting potential for pre- and postnatal nutrient supply. Although milk production increases with litter size, there is no concurrent increase in the synthesis of milk nutrients (1). Mobilization of amino acids (AAs) from muscle protein is a limiting factor in milk protein synthesis. In the mammary gland, the most catabolized AAs are the branched-chain AAs (BCAAs) and arginine (Arg). The BCAAs are oxidized to glutamine (Gln) by the mammary gland (2). Therefore, additional Arg, Gln or both as conditionally essential AA were supplemented to a lactation feed in order to study the impact on sows performance and piglet development.

Methods: Seven days before farrowing,72 sows [DanBred x Piétrain] from a German piglet production site were allocated to one of four treatment groups (n=18) with respect to balanced parity numbers among the treatments. Within 24 h after farrowing the litters were standardized to 13 piglets. The sows were fed restrictedly according to the established feeding regime at the farm, with a minimum of 1 kg at the day of farrowing and a maximum of 8 kg from day 19 to 26 of lactation. The experimental feed was a commercial lactation feed supplemented with either 0.35 % L-Arg, 0.35 % L-Gln, both AA or without supplementation (Control). During the nursing period, piglets did not receive any supplemental feed. With weaning at day 26 of life, they had *ad libitum* access to a commercial prestarter diet and were surveyed for further 14 days. During a total experimental period of 48 days, zootechnical parameters (body weight and condition, feed intake) were assessed weekly. At farrowing, at day 12 and 25, milk samples were taken from 50 % of the sows in order to analyze milk nutrients and AA profile. A two-factorial ANOVA was performed using SPSS ($P<0.05$).

Results: Sows receiving 0.35 % L-Arg showed highest loss in backfat thickness ($P<0.05$), but body weight was not reduced during lactation. The highest weaning weights per piglet were observed in this treatment, but the effect was not significant ($P>0.2$). The piglets from L-Arg-supplemented mothers had higher body weights at day 7 ($P<0.05$) and 14 ($P=0.051$) after weaning. Interestingly, this effect seems to be attributable to the lighter littermates, since an arithmetical block separation by birth weight (1.2 vs. 1.6 kg) showed Arg effects exclusively on the daily gain of lightweight ($P<0.05$) but not on the heavier piglets ($P>0.5$). Piglets with a higher birthweight showed higher weight gains after weaning if their mother was fed the combination of Arg+Gln ($P<0.01$). The nutrient composition of colostrum was affected, but not that of milk samples. The colostrum of the Arg-group contained significantly more fat and lactose, but less protein ($P<0.05$). Supplementation of 0.35 % L-Gln had no substantial effect the parameters described.

Conclusions: It might be assumed that manipulation of milk nutrients by use of L-Arg has a certain window of opportunity, since nutrients were affected in colostrum but not in milk. Supplementation of L-Arg induces a shift from protein to energy production in colostrum, probably with long-term effects on piglets' weight development. Piglets with low birthweights responded stronger to maternal L-Arg supplementation than heavier littermates, indicating differences in AA requirements among different weight categories of piglets.

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* *Freie Universität Berlin Institut für Tierernährung, Königin-Luise-Str. 49, 14195 Berlin, Germany, anna.wessels@fu-berlin.de*

32.

Effect of oral glutamine supplementation during the early neonatal phase on growth, milk intake and plasma metabolites of low birth weight piglets

Einfluss der oralen Glutamin-Supplementierung in der frühen neonatalen Phase auf Wachstum, Milchaufnahme und Plasma-Metaboliten von Ferkeln mit niedrigem Geburtsgewicht

Sciascia Q. L., Li Z., Schregel J., Görs S., Tuchscherer A., Metges C. C.* – Dummerstorf

Low birth weight (L) piglets suffer from higher rates of mortality, and delayed development and growth compared to normal birth weight (N) piglets (1). Maternal milk is thought to provide insufficient glutamine (Gln) for maximal growth of suckling piglets (2). Thus, additional Gln supply may improve piglet growth. The objective was to investigate the effect of oral Gln supplementation, during the early neonatal period, on growth and plasma metabolite profiles of L piglets.

Methods: At birth (0 days postnatal (dpn)), 48 pairs of male German Landrace littermates born to first parity sows, were selected and suckled by their dams. Each pair had one L (1.09 kg ± 0.02, n=48) and one N (1.49 kg ± 0.02, n=48) piglet. At 1 dpn, litters were standardized to 12 piglets/sow and experimental piglets randomly assigned to oral Gln (1g/kg body weight (BW)/day, Gln-L, Gln-N, n=24) or alanine (Ala, 1.22g/kg BW/day; isonitrogenous to Gln, Ala-L, Ala-N n=24) groups, and supplemented throughout the course of the trial. Each dose was made fresh daily and dissolved in 2 ml of water prior to supplementation. To ensure the entire dose was given, syringes were rinsed with a further 2 ml of water, which was also given to the piglets. Piglets were scored for intrauterine growth restriction at birth and weighed daily. Crown-rump length (CRL) and abdominal circumference (AC) were measured at birth (0), 5, 7 and 12 dpn. At 11 dpn piglets were injected with 70% D2O (i.p; 1 g / kg BW) and milk intake calculated (3). Plasma was collected at 5 and 12 dpn to analyze metabolites and milk intake. Data was analyzed using the MIXED procedure of SAS, and where applicable, with repeated measures. Least square means were separated using the Tukey test ($P<0.05$).

Results: Gln-L piglets were heavier than Ala-L piglets at 10 ($P=0.07$), 11 ($P=0.03$) and 12 dpn ($P=0.02$), had a higher milk intake (375.9 vs 327.6 g/kg BW/day, $P=0.02$), were longer at 12 dpn (CRL: 32.9 vs 31.4 cm, $P=0.05$) and wider at 5 dpn (AC: 28.8 vs 26.8 cm, $P=0.01$) than Ala-L piglets. Plasma triglycerides (TG) were lower (5 dpn: 0.9 vs 1.2 mM, $P=0.06$) and plasma urea and non-esterified fatty acids (NEFA) were higher (12 dpn: 3.4 vs 2.7 mM, $P=0.07$ and 401 vs 310 µM, $P=0.06$) in Gln-L compared with Ala-L piglets. Regardless of supplementation L piglets were always lighter, shorter and skinnier than their N littermates. At 5 dpn, Ala-L piglets had a higher concentration of plasma TG and alanine aminotransferase (ALT) activity than Ala-N ($P<0.05$). At 12 d, Gln-L piglets tended to have higher plasma urea (3.4 vs 2.8 mM, $P<0.1$) and significantly higher NEFA levels (401.1 vs 303.5 µM, $P<0.05$) than Gln-N piglets. No other differences were observed.

Conclusions: We conclude that oral Gln supply during the early neonatal period improved L growth compared to Ala piglets, with no observable effect compared to N piglets. At 5 dpn Gln-L piglets had identical levels of plasma TG compared to Ala- and Gln-N piglets, which were all lower than those in Ala-L, suggesting altered lipid metabolism. Additionally, at 12 dpn Gln-L had higher levels of plasma NEFA and urea compared to Ala-L and Gln-N piglets, potentially indicating further altered lipid metabolism, and higher milk intake.

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* *Leibniz Institute for Farm Animal Biology Nutritional Physiology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany, metges@fbn-dummerstorf.de*

33.

Effects of the conservation method of grass forages on protein components of organically produced, raw cows' milk – free amino acids and biogenic amines

Einfluss der Konservierungsmethode von Grünfutter auf die Proteinfraktionen in ökologisch produzierter Milch - freie Aminosäuren und biogene Amine

*Haselmann A., Fuerst-Waltl B., Mayer H. K., Van den Oever S. P., Schmidt A., Zebeli Q., Knaus W. – Vienna

The method of forage conservation could have an effect on cows' energy supply, the utilization of feed protein for milk synthesis, and the proteolytic activity of bacteria in milk. Many of these factors could potentially impact milk protein composition, which is highly relevant for the production of hard cheese. In heavily forage-based feeding systems, the effects of the forage conservation method on milk components were probably stronger than in conventional systems. The objective of the study was to investigate the effects of the conservation method on the daily yield of true protein, non-proteinogenic N (NPN), casein, whey protein, free amino acids (fAA), and biogenic amines (BA) via milk.

Methods: Wilted forages (approx. 55% DM) of a grass dominated sward (1st cut) were obtained from the same field and harvested at the same time (28 h after the cut). A single loading wagon picked up the windrows alternately, either for ensiling in a horizontal silo or barn-drying with a dehumidifier. A feeding trial with 18 Holstein cows started 9 month later. Cows were divided into 2 feeding groups by previous milk yield (MY; 30.0 ± 5.9 kg), body weight (717 ± 53 kg), DIM (171 ± 104 d), and parity (3.8 ± 2.1). Groups were either fed with silage (6.21 MJ NE_L, 124 g CP, 117 g WSC, and 477 g NDF per kg DM) or hay (6.15 MJ NE_L, 114 g CP, 180 g WSC, and 485 g NDF per kg DM). In addition, each cow received 3.6 kg (DM) of concentrate (7.52 MJ NE_L, 288 g CP, 131 g starch, and 251 g NDF per kg DM). Concentrate contained soybean cake, sugar beet pulp, wheat bran, molasses, and vitamins and minerals in a ratio (DM) of 51:23:18:3:5, respectively. Data collection started after a 2-week adaptation period of cows to the respective diet. On three days of the data collection period (d 1, 10, and 17), individual milk samples were collected which were derived from 2 consecutive milkings. For proximate analyzes, samples were pooled for each cow. Protein components (N × 6.38) were determined according to the current IDF/ISO standards (*Kjeldahl*): Total N (1), non-casein N (2), NPN (3). Whey protein N was calculated from the difference between non-casein N and NPN, and casein N from total N and non-casein N. Free amino acids and BA were analyzed according to (4) using UHPLC.The statistical model (proc glm SAS 9.4) included the treatment as fixed effect and DIM as a covariable.

Results: Daily yield (g/d) of true protein, NPN, casein, and whey protein did not differ ($P > 0.10$) between treatments. Despite the lower MY of silage-fed cows (28.1 ± 7.05 *vs* 29.4 ± 6.80 kg), they secreted a higher amount of fAA into milk (+ 697.0 mg/d; $P = 0.074$) than hay-fed cows. This elevation stemmed from increased amounts (mg/d) of Glu (+ 488; $P = 0.078$), Gly (+45.3; $P = 0.056$), Gln (+41; $P = 0.013$), Asp (+ 37; $P = 0.113$), Ser (+10.9; $P = 0.007$), Tyr (+5.2; $P = 0.055$), and Asn (+5.1; $P = 0.014$). There was no treatment effect ($P > 0.10$) on the production of free essential AA in milk.

Furthermore, silage-fed cows secreted higher amounts of spermine (10.9 vs 6.1 mg/d; $P = 0.016$) into milk as compared to cows on hay. In contrast, intermediate products (i.e. BA) of the spermine synthesis such as spermidine, agmatine, and putrescin were not affected ($P > 0.226$) by the treatment. Daily amount of other BA in milk such as colamine, histamine, ethylamine, octopamine, tyramine, methylbutylamine, and phenylethylamine did not differ ($P > 0.116$) among treatments.

Conclusions: The forage conservation method affected the secretion of free non-essential AA and polyamines (i.e. spermine) into milk. The greater production of fAA via milk in silage-fed cows reflects a greater uptake of AA and energy by the mammary gland. The higher amount of spermine in the milk of silage-fed cows indicates an elevated provision of relevant precursors (Arg, Orn, Met) for the spermine biosynthesis.

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* *University of Natural Resources and Life Sciences (BOKU) Department of Sustainable Agricultural Systems, Gregor Mendel Strasse 33, 1180 Vienna, Austria, andreas.haselmann@boku.ac.at*

34.

Suitability of dual-energy X-ray absorptiometry for determination of nitrogen content of pigs for genetic and nutrition studies

Eignung der Dual-Energie-Röntgenabsorptionsmessung zur Bestimmung des Stickstoffgehalts von Schweinen für Genetik- und Ernährungsstudien

*Kasper C., Ruiz-Ascacibar I., Stoll P., Bee G. – Posieux

Nitrogen efficiency, i.e. the proportion of nitrogen uptake in the body to the total nitrogen intake is an ecologically and economically important phenotype for the genetic selection of pigs. Preliminary analyses suggest a heritability of 32% and 16% for nitrogen efficiency in the empty body and carcass (1), respectively. However, a reliable estimation of genetic parameters or genome-wide association studies requires a large number of phenotyped individuals. Traditionally, the lean meat content of the carcass or the empty body is determined via dissections, which suffer from operator bias, or chemical analysis, which is labour-intensive and expensive. Thus, methods with a potential for high throughput are needed. These methods will also help develop precision feeding strategies. Here, we explore whether dual-energy X-ray absorptiometry (DXA) is a suitable alternative in terms of accuracy and precision in a Swiss Large White pig population. DXA scans provide a measure for body composition such as lean meat content (2), from which the nitrogen content can be derived. However, the values obtained by DXA are systematically biased because they are an indirect measure and therefore require calibration. Hence, prediction equations are needed to estimate the true nitrogen content (as obtained by chemical analysis as the golden standard).

Methods: We obtained measurements of both nitrogen content by chemical analysis as previously described (3) and lean meat content with DXA (GE Lunar iDXA with pencil beam) of both live animals and carcasses of the same 68 entire males (6 pigs with live weight of 20 kg, 18 with 60 kg and 44 with 100 kg). . We derived linear equations to predict nitrogen content from DXA lean tissue measurements and we determined R^2 and RMSE as measures of precision and accuracy.

Results: We found that chemically determined nitrogen content can be predicted by DXA measurements with high accuracy and precision. The linear equation to estimate nitrogen content of the live pig is and for the carcass. Both equations yield high precision of estimates (R^2=0.982 and R^2=0.983 for live pig and carcass, respectively) and values could be predicted with little error as indicated by RMSE=4.7% and RMSE=4.4% of the mean of the chemically determined nitrogen content).

Conclusions: Due to the high accuracy and precision of predicted nitrogen contents of body and carcass, we conclude that this method provides a practicable and non-invasive option for determining nitrogen content in empty body as well as the carcass in a high throughput fashion that is needed for genetic studies. The applicability of prediction equations is expected for females and castrates, but awaits confirmation. Since potential breeding animals do not have to be sacrificed, DXA could aid performance testing and the selection of parent individuals for breeding because breeding values can be estimated directly for dams and sires and not from relatives, which will increase accuracy and accelerate genetic gain. Non-destructive methods to determine body composition precisely will aid the development of precision feeding.

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* *Agroscope Animal Production Systems and Animal Health, Tioleyre 4, 1725 Posieux, Switzerland, claudia.kasper@agroscope.admin.ch*

35.

Relationship between heart frequency, activity, velocity and size of saddle horses and ponies under practical conditions

Beziehungen zwischen Herzfrequenz, Aktivität, Geschwindigkeit und Größe bei Reitpferden und Ponys unter Praxisbedingungen

*von Reitzenstein C., Gerth N., Kienzle E. – Oberschleißheim

Heart rate measurement in horses can be used to predict energy expenditure during exercise (see 1). There are also correlations between heart rate and velocity. Measurements have been carried out mostly in highly trained sports horses under defined conditions. We measured heart rate, velocity and activity under field conditions in ponies and horses to investigate i) whether at moderate velocity data from highly trained horses are similar to those of animals with a lower level of training, ii) whether interspecies allometric considerations are valid within the species *equus caballus* i.e. how data from tall horses can be transferred to ponies (see 1) and iii) to obtain data on velocity, heart rate and energy expenditure during typical activities in horses and ponies.

Methods: Eighty-four animals took part in the study. The participants were grouped according to withers' height (ponies P ≤148 cm; small horses K ≤160cm; tall horses G >160cm). The horses and ponies were tacked with a belt under the saddle or girth, specially built for horses, which measures the heart rate and a watch GPS system to measure the velocity (POLAR V800, polar watch). An accelerometer (Actical) which measures the activity in counts/second was put on the left front leg. The horses were measured either during riding in an arena, during riding out in the fields or training or during lunging. There were 112 observations in total. Means were compared by ANOVA, and Holm-Sidak post hoc test ($p<0.05$ was condiered as significant. Linear regressions were calculated between parameters.

Results: Velocity of the horses depended on the gait, the type of activity as well as the size of the horse. When horses where ridden in the field they were faster than when ridden in an arena at the same gait. During lounging they were even slower. There was a significant correlation between velocity and heart rate ($r^2=0.32$; $p< 0.01$). Ponies and smaller horses had higher heart rates at lower velocity, especially in fast gaits. For instance, ponies running with a velocity of 6 m/s had a comparable heart rate of 180-200 bumps per minutes as tall horses running with a velocity of 12 m/s. During lunging there were higher heart rates at lower velocity. Activity counts correlated strictly with the heartrate and did not show any effect of size or type of exercise ($r^2=0.74$; $p<0.01$). Stride length, however, depended on size, velocity and type of exercise. For instance, in canter/gallop during lunging tall horses had a stride length of 2.5±0.5 m, during ridden work in an arena of 3.3±1.4 m and in the field of 4.7±1.3 m.

Conclusions: Given the fact that cardiac output depends linearly on body weight (2Holt et al., 2004) it can be assumed, that the oxygen consumption based on the body mass at similar heart rates is comparable in small and tall horses. The relationship between heart rate and velocity, however, is different: Velocity at the same heart rate is lower in ponies and small horses than in tall ones. This finding suggests that interspecies allometric considerations (see 1) are valid in horses. For practical considerations the type of activity i.e. in an arena, out in the fields or lunging is important for assessment of energy requirements for work.

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* *LMU München Chair of Animal Nutrition and Dietetics, Schönleutner Str. 8, 85764 Oberschleißheim, Germany, caro.reitzenstein@googlemail.com*

36.

Influence of dietary energy concentration on the empty body composition of growing Fleckvieh bulls

Einfluss der Energiekonzentration in der Ration auf die Leerkörperzusammensetzung von wachsenden Fleckviehbullen

*Honig A., Inhuber V., Spiekers H., Windisch W., Götz K.-U., Ettle T. – Grub / Freising

The performance potential of Fleckvieh (German Simmental) fattening bulls has been improved by selective breeding during past decades. This might have also affected the carcass tissue composition as well as chemical body composition and hence energy and nutrient requirements of animals during fattening. In order to reevaluate the deposition of energy and nutrients and the chemical and body tissue composition of growing Fleckvieh bulls of modern type, a feeding and slaughter experiment was conducted. The present abstract presents data on empty body tissue composition of bulls slaughtered in different weight categories after feeding diets with varying energy concentrations.

Methods: 72 Fleckvieh bulls (age: 42 d, body weight (BW) 80 kg) were fed with restricted amounts of milk replacer (120 g/l) and a concentrates/hay-based total mixed ration (TMR) until weaning at an average BW of 121 kg and subsequently on a TMR based on maize silage and concentrates for ad libitum intake. The fattening period was initiated at an average BW of 225 kg, when the bulls were allocated to a normal and a high energy treatment group with 11.6 and 12.4 MJ ME/kg DM, respectively. Individual feed intake was recorded daily and BW was determined in four-weeks intervals. The bulls were slaughtered in five weight groups with 120 (n=8), 200 (n=10), 400 (n=18), 600 (n=18), and 780 kg (n=18) final live weights. During slaughtering and carcass processing, the empty body weight was determined as final live weight minus the contents of urinary bladder and gastrointestinal (GI) tract and the whole empty body was anatomized to body tissues as hide, blood, organs, empty GI tract, body fat, muscle, bone and tendon. Statistical analysis was performed using Proc GLM of SAS (Version 9.3). Results are shown in ranges and standard error and were compared by SNK method with values of $p<0.05$ stated as significant.

Results: The empty body weights of weight groups 120, 200, 400, 600, and 780 kg were 104, 176, 370, 553 and 734 kg, respectively. Since there were only minor effects of dietary energy concentration on empty body tissue composition in normal and high energy treatment groups, the combined results of both animal groups are shown. Muscle and tendon percentage of empty body weight, with average of 42.9 % ±0.5 and 4.2 % ±0.1, respectively, did not vary between weight groups. During growth, the percentage of blood, organs, GI tract, and bone decreased ($p<0.05$; blood: 6.0-4.0 % ±0.1; organs: 7.2-5.7 % ±0.1; GI tract: 7.4-3.9 % ±0.2; bone: 19.0-11.1 % ±0.2), while hide and body fat percentage increased ($p<0.05$) from 9.2 to 10.5 % ±0.2 and 3.7 to 18.5 % ±0.6, respectively.

Conclusions: The empty body compositions of modern type Fleckvieh bulls corresponded widely to literature data from past decades (1). During growth, the amount of body fat increased mainly at the expense of bone tissue. A decrease of muscle tissue in higher weight classes could not be observed. Variations in dietary energy concentrations within margins found under practical conditions did not alter the body composition to a relevant extent.

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* *Bayerische Landesanstalt für Landwirtschaft Institut für Tierernährung und Futterwirtschaft, Prof.-Dürrwaechter-Platz 3, 85586 Grub, Germany, aniela.honig@lfl.bayern.de*

37.

Relationship between the energy balance and the contents of lauric and myristic acid in hair of Holstein and Simmental cattle during the transition period

Beziehung zwischen der Energiebilanz und den Laurin- und Myristinsäuregehalten in Haaren von Holstein und Fleckvieh-Kühen in der Transitphase

*Möller R., Arends D., Dannenberger D., Brockmann G. A. – Berlin / Dummerstorf

Previously, we have shown that primiparous cows with a short interval from calving to conception and a high milk protein yield (1) as well as cows with a high energy utilisation in lactation weeks 1 to 6 (2) had higher contents of specific fatty acids, like lauric acid, in hair in lactation week 8. Until now, it has not been examined if hair sampling during early lactation would also reflect the energy balance of cows in the transition period. Therefore, the aim of this study was to investigate the relationship between energy balance around calving and the content of lauric and myristic acids in the hair of cows in week 4 of lactation.

Methods: For the study, 125 Simmental and 39 German Holstein cows from four farms were used. All farms were equipped with feeding systems to measure individual daily feed intake. The lactation number varied from 1 to 9. All cows were fed the same diet before calving. After calving, cows were assigned to one of four feeding groups: (1) moderate energy concentration of roughage (6.1 MJ NE_L/kg DM) and moderate amount of concentrates (150 g/kg ECM), (2) moderate energy concentration of roughage (6.1 MJ NE_L/kg DM) and high amount of concentrates (250 g/kg ECM), (3) high energy concentration of roughage (6.5 MJ NE_L/kg DM) and moderate amount of concentrates (150 g/kg ECM), (4) high energy concentration of roughage (6.5 MJ NE_L/kg DM) and high amount of concentrates (250 g/kg ECM). Feeding groups 3 and 4 were kept in all farms, groups 1 and 2 in two farms only. The energy balance (MJ ME) was defined as the difference between energy intake and energy requirements for maintenance, gestation, growth and milk production. To characterise the transition period, we calculated the cumulative energy balance for 4 periods: -3 to -2, -3 to -1, -2 to -1, and 1 to 2 weeks before or after calving, respectively. Since the fatty acid content in the hair of an animal reflects the metabolism of the previous two to three weeks, we examined hair in lactation week 4. The hair samples were shaved from the left ventral side of the foreleg. Hair lipids were extracted from 200 mg cleaned and mill-ground hair with a fatty extraction kit (3). After methylation of fatty acids, the fatty acid methyl esters were analysed using gas chromatography. Pearson's correlation coefficients were calculated over all data without considering breed and lactation number. Multiple linear regression was used to estimate the effects and effect size of lauric and myristic acids on the energy balance. The linear regression model associated energy balance with lauric and myristic acids as predictors. Season, lactation number, breed and feeding group after calving were included as fixed effects. The corrected Akaike information criterion was used for defining the best fitted model. The level of significance was $P < 0.1$.

Results: Significant negative correlation was found between energy balance in the periods of weeks -3 to -2 and -3 to -1 before calving and the content of lauric ($r = -0.17$; $P < 0.07$) and myristic acids ($-0.24 \leq r \leq -0.17$; $P < 0.05$) of hair samples from lactation week 4, respectively. Although the model fit was improved when including lauric and myristic acids into the model, only the energy concentration of roughage had a significant influence on the energy balance in lactation weeks 1 to 2 ($P = 0.05$). The energy balance was inferior in cows on a high to cows on a moderate energy concentration of roughage.

Conclusions: Higher contents of lauric and myristic acids in the hair of cows in lactation week 4 are associated with a better beneficial energy supply and utilisation of a cow before calving. Due to the weak correlation coefficients, a farm based approach including additional phenotypes around calving like changes of body condition scores and blood parameters is suggested to further confirm and refine the relationship between energy balance and lauric and myristic acids in the hair.

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* *Humboldt-Universität zu Berlin Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences, Berlin, Germany, ramona.moeller@agrar.hu-berlin.de*

38.

Effect of feed deprivation on plasma endocannabinoid concentrations in dairy cows

Einfluss eines Futterentzugs auf die Plasma-Endocannabinoidkonzentration bei Milchkühen

*van Ackern I. M., Tuchscherer A., Kuhla B. – Dummerstorf

Endocannabinoids (EC), specifically anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are involved in the regulation of food intake and energy homeostasis (1). Increased EC production from phospholipid membranes in the periphery and the brain enables fast adaptation to negative energy balance in rodents (2). However, little is known about regulation of the EC tone in dairy cows. Therefore, the objective of the present study was to investigate whether an energy deficit provoked by short term fasting increases, and if refeeding normalizes plasma AEA and 2-AG concentrations.

Methods: Seven lactating Simmental cows in 1st-10th lactation (123-390 days in milk) with a mean body weight of 753 ± 52 kg were milked twice daily with an average milk yield of 17.6 ± 6.7 l per day. The cows were kept under loose housing conditions with ad libitum feeding of a total mixed ration consisting of corn silage (55.8%), straw (8.6%), concentrate (33.9%) and minerals (1.7%) with a metabolizable energy (ME) content of 11.5 ± 0.1 MJ/kg. Feed intake was measured electronically using Insentec feeding troughs. For feed deprivation, feeding troughs were emptied and access was blocked from 07.00 am to 02.00 pm. At 02.00 pm troughs were refilled and cows were given back access to feed for ad libitum intake. Blood samples were obtained from the tail vein directly before the deprivation period (07.00 am), after 7 h of feed deprivation (02.00 pm) and in the morning after refeeding (07.00 am). Blood samples were collected in K_3-EDTA tubes and immediately placed on ice, before centrifugation (1.568 *g*, 20 min, 4°C) and storage of plasma at -80°C. Analysis of plasma AEA and 2-AG was performed on a Waters ACQUITY UPLC-MS/MS system. Statistical analysis was carried out using the SAS software for Windows, version 9.4 (Copyright, SAS Institute Inc., Cary, NC, USA). Data were analyzed by ANOVA with repeated measures of time and pair-wise comparisons using the Tukey-Kramer test. Statistical significance level was considered at $P < 0.05$.

Results: Plasma 2-AG concentrations increased after 7 hours of feed withdrawal relative to the basal tone ($P < 0.05$) and decreased after refeeding ($P < 0.05$). However, no significant differences in plasma AEA concentrations were observed.

Conclusions: The results suggest that 2-AG is involved in the immediate response to changes in energy balance, whereas AEA is not reacting to short-term feed deprivation.

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* *Leibniz Institute for Farm Animal Biology (FBN) Institute of Nutritional Physiology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany, vanackern@fbn-dummerstorf.de*

39.

The effect of dietary Ca supplementation on precaecal $InsP_6$ disappearance and P digestibility in broiler chickens depends on the Ca source

Zum Einfluss der Ca-Supplementierung auf das praecaecale $InsP_6$-Verschwinden und die praecaecale P-Verdaulichkeit bei Boilern

*Krieg J., Chi Y. P., Feuerstein D., Siegert W., Rodehutscord M. – Stuttgart / Lampertheim

High levels of $CaCO_3$ in the diet are known to negatively affect precaecal (pc) $InsP_6$ disappearance and P digestibility in broiler chickens. However, effects of using different sources of Ca that have different effects on gastrointestinal pH are hardly studied. The present study was conducted to investigate whether the exchange of $CaCO_3$ by Ca formate and the supplementation of formic acid to $CaCO_3$ supplemented diets alters pc $InsP_6$ disappearance and P digestibility.

Methods: Six maize-based diets were formulated to achieve two different Ca levels (5.6 and 8.2 g/kg DM) by inclusion of either $CaCO_3$, $CaCO_3$+formic acid, or Ca formate (Ca supplements). Each diet was mixed with and without supplementation of a hybrid 6-phytase (1500 FTU/kg feed) and pelleted. No mineral P was supplemented in the diets. Titanium dioxide was included as indigestible marker (0.5 %). All nutrients except P were formulated to meet or exceed the recommendations of the GfE (1). The birds were kept on wood shavings and were offered a commercial starter diet until day 16. From day 16 to 21/22, the animals were housed on perforated floor and provided the experimental diets (6 pens per diet, 15 animals per pen, a total of 1080 animals). On day 21/22, animals were sacrificed and digesta from the terminal half of the ileum was flushed out using ice-cold deionized water and pooled on a pen basis. Samples were analyzed for the concentrations of P, InsP isomers, and Ti. Results were statistically analyzed considering Ca source, Ca level, phytase supplementation, and interactions between these factors as fixed effects, and the pen as a random effect at a significance level of $\alpha = 5\%$ (proc mixed of SAS 9.4).

Results: Precaecal $InsP_6$ disappearance and P digestibility was significantly increased by phytase supplementation in all diets. The pc $InsP_6$ disappearance was significantly affected by the Ca level × phytase and Ca source × phytase interactions; it was significantly decreased by an increase in the Ca level without phytase (low: 41%, high: 32%, $P<0.001$), but unaffected in phytase supplemented diets (low: 81%, high: 80%, $P=0.469$). While an increase in the Ca level lead to a significant reduction in pc $InsP_6$ disappearance in $CaCO_3$ and Ca formate supplemented diets (63% to 59%, $P=0.047$ and 57% to 47%, $P<0.001$), pc $InsP_6$ disappearance was unaffected in $CaCO_3$+formic acid supplemented diets (63 and 62%, $P=0.784$). The pc P digestibility was significantly affected by the Ca source × Ca level interaction and by phytase. The increase in Ca level reduced pc P digestibility more when Ca formate was used (58% to 46%, $P<0.001$) compared to $CaCO_3$ and $CaCO_3$+formic acid (60% to 53% and 61% to 54%, $P<0.001$). Overall, phytase supplementation significantly increased P digestibility by 28 percentage points to 69% ($P<0.001$).

Conclusions: Ca from Ca formate affected pc $InsP_6$ disappearance and P digestibility more than Ca from $CaCO_3$. This might be attributed to the higher solubility of Ca-formate and thus, more Ca^{2+} available for the formation of Ca-$InsP_6$ complexes. Supplementation of formic acid together with $CaCO_3$ increased pc $IncP_6$ disappearance, probably by decreasing the intestinal pH. The results demonstrate that it is important to distinguish between Ca sources when effects of Ca and phytases on nutrient digestibility are studied.

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* *University of Hohenheim Institute of Animal Science, Emil-Wolff-Str. 6-10, 70599 Stuttgart, Germany, jochen.krieg@uni-hohenheim.de*

40.

Microbial changes in the crop and the ileum under the influence of dietary Ca supplementation in broiler chickens

Untersuchung von Veränderungen in der Mikrobiota des Kropf und Ileums unter dem Einfluss der Ca-Versorgung bei Broilern

*Borda-Molina D., Krieg J., Feuerstein D., Rodehutscord M., Camarinha-Silva A. – Stuttgart / Lampertheim

Previous studies based on sequencing data revealed that the addition of monocalcium phosphate increased the abundance of *Lactobacillus taiwanensis,* a beneficial bacterium in the gastrointestinal tract of chickens (1). The aim of the present study was to follow-up on the microbial ecology changes when different concentrations and sources of Ca were added to the diet. Relationships between the presence or absence of specific microbial groups and digestibility traits were also studied.

Methods: Six low-P maize-based diets were formulated to achieve two different Ca levels (5.6 and 8.2 g/kg DM) by the inclusion of either CaCO3, CaCO3+formic acid, or Ca formate (Ca supplements). Each diet was supplemented with and without a hybrid 6-phytase (1500 FTU/kg feed). From day 16 to 21, each experimental diet was provided to 6 groups of 15 broilers. On day 21, birds were stunned by a gas mixture and euthanized by CO2 exposure. Digesta from the crop and the ileum were collected, pooled on a pen basis and stored at -20°C. Total nucleic acids were extracted and then subjected to 16S rRNA gene amplicon sequencing. Bioinformatics analysis followed the mothur pipeline (2), and reads were assigned using the Seqmatch function (RDP website). Microbial genomes from these assignations were used to perform functional predictions (3). The multivariate statistical analysis was carried out to study both taxonomy and functional datasets.

Results: A significant effect of diet was observed in the microbial ecology of the crop and ileum ($P < 0.05$). The main effects, including Ca level, Ca source, and phytase were significant, but not the interactions between them. Dominance of the genus *Lactobacillus* was observed in both environments. The supplementation of only CaCO3, with low Ca level, and either presence or absence of phytase addition, induced a higher microbial percentage similarity between two diets (diet low Ca with CaCO3 and diet low Ca with CaCO3 and phytase). *Streptococcus alactolyticus* was driving this relationship, due to an increase in its relative abundance in the above-mentioned diets. Functional predictions computed based on bacterial abundances, displayed in the crop, mainly genes assigned to metabolic activities (on average 98%). Meanwhile, the ileum showed more communication with the host since it contains, on average, 20% of the gene abundance assigned to the broad classification level of environmental- and genetic-information processes. In the crop, addition of phytase increased the abundance of *Lactobacillus johnsonii*, while *Lactobacillus gallinarum* was more abundant in the absence of phytase. Overall, measured pH values did not show a clear influence on microbiota composition. Only in the crop, a significant negative correlation was observed with one sequence identified as *L. gallinarum*. The level of Ca supplementations and the Ca sources (CaCO3 and CaCO3+formic acid) were causing this correlation. In the ileum, a positive correlation was observed between the supplementation of phytase and *L. johnsonii*. Also in the ileum, *Lactobacillus vaginalis* was more abundant in diets without phytase but positively correlated to the presence of InsP5 isomer

Conclusions: The supplementation of CaCO3+formic acid and Ca formate with or without phytase caused a similar overall microbial distribution. Nevertheless, supplementation of CaCO3 alone had a different effect on this distribution. The most abundant assigned microorganisms increased or decreased their abundance depending on phytase addition, being this an indication of the high interaction between the degrading activity of the enzyme and the microbial interactions in the digestive tract. Microbiota composition was affected by the pH value in the crop, while prececal measurements had more interactions with ileum microbiota.

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* *University of Hohenheim Institute of Animal Science, Emil-Wolff-Str. 6-10, 70599 Stuttgart, Germany, danielbm@uni-hohenheim.de*

41.

Effects of low dietary phosphorus and zinc levels on the zinc status of laying hens

Zum Einfluss einer niedrigen alimentären Phosphor- und Zinkversorgung auf den Zinkstatus von Legehennen

*Brugger D., Weller A., Puntigam R., Slama J., Windisch W. – Freising / Vienna

Zeller et al. (1) have demonstrated efficient phytate hydrolysis of ~75% until the end of the ileum of broilers fed low dietary P. Hence, the dietary P supply potentially affects the bioavailability of phytate associated divalent cations such as zinc. The present study compared the effects of feeding high and low dietary P in combination with high and low dietary Zn, respectively, to investigate the interaction of these parameters in the modulation of the zinc status of laying hens.

Methods: 48 fully grown laying hens were kept in 24 boxes housing each one Lohmann Brown and one Lohmann White hen (initial average body weight 2134 ± 222 g and 1809 ± 82.1 g), respectively. Laying performance was 1.00 egg*(hen*day)$^{-1}$ of ~65 g/egg. The experiment comprised a 14d acclimatization and an 8d experimental phase. During acclimatization, animals were fed according to recommendations except for P (AMEn 11.4 MJ/kg, XP 20.8%, Lys 1.23%, Met 0.37%, total Ca 38g/kg, total Zn: 130mg/kg by adding 100 mg Zn/kg from $ZnSO_4$*$7H_2O$). P supply was split into two levels (low P: only native P: 3.67g/kg; normal P: 8.42g/kg by adding 4.75g P/kg from mono-Ca-phosphate). During the experimental phase, the P feeding regimen was continued and was additionally split into two levels of Zn (low Zn: only native Zn 27.3mg/kg, normal Zn as during acclimatization phase). The respective diets were added with 0.1% of TiO_2 as indigestible marker. During experimental d8 excreta were collected boxwise and animals were then sacrificed to collect blood plasma, left femur and liver. Zootechnical performance was recorded, feeds and excreta were analysed for Zn, P, and TiO_2. Liver, bone and blood plasma were analysed for Zn. Data analysis comprised 3-way ANOVA (P, Zn, Genotype; SAS 9.4) and level of significance was set at $p<0.05$.

Results: Zootechnical parameters, egg yield and egg quality were not affected by the dietary treatment except for a transient reduction of egg weight in the low P group at the end of the acclimatization phase ((-1.66 g, P = 0.01). Low dietary Zn reduced plasma Zn, liver Zn and apparently retained amounts of dietary Zn (-1.4 mg/L, -1.4 mg/kg, -5.8 mg/kg with P = 0.02, 0.03 and <0.0001, respectively) irrespective of the dietary P supply. Low P reduced femur Zn by 60.2 mg/kg ash (P = 0.02), irrespective of the dietary Zn supply (P = 0.25).

Conclusions: Low Zn supply induced subclinical Zn deficiency within 8 days (reduced Zn in blood plasma and liver, reduced apparent Zn retention). This is in agreement with earlier data in pigs (2). However, bone Zn did not respond to dietary Zn supply but to P supply. Presumably, animals mobilized bone matter in response to a widened Ca:P dietary ratio which overruled effects of Zn supply on bone Zn. The interaction between alimentary Ca-P-supply and the body Zn status deserves more attention in future studies.

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* *Technical University of Munich Chair of Animal Nutrition, Liesel-Beckmann-Straße 2, 85354 Freising, Germany, daniel.brugger@wzw.tum.de*

42.

Influence of luminal sodium availability and menthol prefeeding on ruminal calcium transport in growing sheep

Einfluss von luminaler Natriumverfügbarkeit und einer Vorfütterung mit Menthol auf den Kalziumtransport im Pansen heranwachsender Schafe

*Geiger S., Patra A. K., Schrapers K. T., Aschenbach J. R. – Berlin / Kolkata

A large amount of Ca^{2+} is absorbed in the forestomach. There is still ambiguity about the pathway and involved transport proteins of Ca^{2+} absorption in the rumen. On the apical cell pole, the involvement of a Ca^{2+}/H^{+} exchanger and/or a nonselective cation channel of the transient receptor potential (TRP) family were proposed to mediate the rate-limiting step in transepithelial Ca^{2+} absorption. Menthol, which acts as an agonist for TRPV3, increased Ca^{2+} flux rates across isolated ruminal epithelium of cattle *ex vivo* [1]. In a feeding study on mid-lactating cows, menthol supplementation led to a significant higher Ca^{2+} plasma concentration [2]. Thus, the purpose of this research was to evaluate the functional involvement of menthol-stimulated conductances in ruminal Ca^{2+} absorption and the possibility of an additional involvement of Ca^{2+}/H^{+} exchange.

Methods: Twenty-four growing Suffolk sheep were fed hay *ad libitum* and distributed equally to three different concentrates. The corn-based pelleted concentrate of the control group contained no plant bioactive lipid compounds (PBLC), whereas the other two groups received the same concentrate with 80 mg/d (PBLC-L) or 160 mg/d (PBLC-H) of PBLC with menthol as a major component (90%). After 28 days of feeding, ruminal epithelia were obtained and short-circuit current (I_{sc}), tissue conductance (G_t) and unidirectional and net flux rates of Ca^{2+} were measured in the absence of mucosal Mg^{2+} in Ussing chambers. To distinguish between the proposed transport proteins, the effect of the presence and absence of mucosal Na^{+} on Ca^{2+} absorption was tested. In the absence of Na^{+}, protons cannot be extruded via the apical Na^{+}/H^{+} exchanger, which should increase the activity of Ca^{2+}/H^{+} exchange, resulting in an increased net Ca^{2+} flux rate. Data were analyzed using the PROC MIXED procedures of SAS and polynomial contrasts for linear and quadratic effects.

Results: Baseline I_{sc} of control animals in the presence of luminal Na^{+} amounted to 12.3 ± 1.13 $\mu Eq \cdot cm^{-2} \cdot h^{-1}$ and was increased by PBLC prefeeding in a dose-dependent manner by 63% and 71% in PBLC-L and PBLC-H, respectively. Baseline G_t (3.8 ± 0.123 $mS \cdot cm^{-2}$) was not influenced by treatment ($P > 0.05$). The omission of luminal Na^{+} decreased both I_{sc} and G_t ($P < 0.001$), G_t decreased by 28%, 27% and 21%, I_{sc} decreased by 193%, 146% and 142% in control, PBLC-L and PBLC-H, respectively. Ca^{2+} net flux rates increased in a quadratic manner with PBLC prefeeding ($P < 0.05$) with highest flux rates for PBLC-L animals. The omission of Na^{+} decreased mucosal-to-serosal Ca^{2+} flux rate ($P < 0.001$), resulting in a decrease of Ca^{2+} net flux rate by 29%, 34% and 34% in control, PBLC-L and PBLC-H, respectively ($P < 0.001$).

Conclusions: The decrease of Ca^{2+} net flux rates with the omission of luminal Na^{+} challenges the functional expression of a putative Ca^{2+}/H^{+} exchanger in the rumen. Menthol prefeeding increases I_{sc} and active, transcellular Ca^{2+} absorption in the rumen, most likely by stimulation of TRPV3.

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* *Freie Universität Berlin Institute of Veterinary Physiology, Oertzenweg 19b, Haus 11, 14163 Berlin, Germany, sebastian.geiger@fu-berlin.de*

43.

The increase of faecal Ca excretion with increasing faecal dry matter excretion in dogs is an independent per se effect of faecal dry matter excretion

Die mit steigender faecaler Trockensubstanzausscheidung zunehmende faecale Ca-Ausscheidung beim Hund ist ein unabhängiger per se Effekt der faecalen Trockensubstanzausscheidung

*Kienzle E., Hofmann C., Dobenecker B. – Oberschleißheim

In two previous studies (1,2) an increase of faecal Calcium (Ca) excretion with increasing faecal dry matter (DM) excretion was demonstrated by multiple regression calculations with Ca intake and faecal DM excretion as independent and faecal Ca excretion as dependant variable. In the first study the increase of faecal DM excretion was exclusively due to addition of purified fibre (1). In the second retrospective study digestion trials with prepared pet food were evaluated (2). A high faecal DM excretion could then be due to an increased percentage of bone material a potential confounding factor. A strong influence of faecal DM excretion on faecal Ca excretion would be an important factor to take into account for the calculation of Ca requirements. Therefore, In the present study dogs were fed two levels of the same low Ca basal diet with the same amount of Ca added to ensure a different faecal DM excretion in otherwise identical diets.

Methods: Eight adult foxhound-mix (FBI)dogs (24-32 kg body weight; BW) were available for the study. A basal diet of cooked pork meat, cooked rice and gelatin was prepared. In the first trial, the dogs were fed this diet to maintain their BW, in the second trial the food allowance was increased (DM intake 8.5±0.7 g/kg BW and 12.6±1.2 g/kg BW respectively). Mineral supplements were added individually to achieve a constant and high intake of Ca, respectively (226 mg Ca/kg BW, Ca/P 1.3/1). Digestion trials (10 days adaptation, 5 days total faecal collection) were carried out. Means were compared by paired t-test, linear regressions were calculated between parameters.

Results: Digestibility of DM averaged 87 % in both trials. Faecal DM excretion was rather low, the difference between the trials, however, was highly significant and increased from 1.1±0.3 to 1.7±0.2 g/kg BW. The same was true for faecal Ca excretion (185±34 and 233±22 mg/kg BW respectively). Apparent digestibility of Ca decreased from 18 to -3 %. Apparently digested Ca decreased from 41 to - 8 mg/kg BW. There was a highly significant relationship between faecal DM excretion (x; g/kg BW) and faecal Ca excretion (y; mg/kg BW; r^2=0.92; y=105x+62; p<0.01).

Conclusions: The increase of faecal Ca excretion with increasing DM excretion confirms that DM intake and digestibility are important factors for the calculation of canine Ca requirements. This can be highly relevant in special diets with a lower digestibility such as weight loss diets, vegan food, or in case of a high feed intake (work, lactation). In trials on Ca metabolism a strict control of faecal DM is necessary. The study supports previous hypotheses that active intestinal Ca absorption plays a minor role in canine Ca homeostasis (3). This may be a general feature of a carnivorous feast and famine lifestyle.

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* *Ludwig-Maximilians-Universität München, Chair of Animal Nutrition and Dietetics , Schönleutner Str. 8, 85764 Oberschleißheim, Germany, kienzle@tiph.vetmed.uni-muenchen.de*

44.

Influence of phosphorus source on faecal calcium and phosphorus excretion in dogs

Einfluss der Phosphorquelle auf die faecale Calcium- und Phosphorausscheidung beim Hund

*Böswald L. F., Herbst S., Dobenecker B. – Oberschleißheim

Dietary phosphorus (P) sources can be grouped in "organic" (P_o) and inorganic (P_i) compounds, differing in solubility (1), which is a precondition for absorption from the gastrointestinal tract. There are differences in P homeostasis depending on P source. The aim of this study was to use data from a high P trial (2) to test whether delta, the difference between dietary and faecal calcium (Ca) / P ratios, can be used to distinguish between dietary P sources.

Methods: In the trial by Herbst & Dobenecker (2), adult beagle dogs (n = 8, 14±1 kg body weight) were fed experimental diets (64 % rumen, 32 % rice, 4 % casein) with P 5 times the NRC requirement, differing in P source (2). P_o sources were bone meal (BM) in two particle sizes (<1mm and >1mm) and poultry meal (PM; diet composition: 57% poultry meal, 43% rice). P_i sources were NaH_2PO_4, KH_2PO_4, $Ca(H_2PO_4)_2$ and $CaHPO_4$. The dietary Ca/P ratio was 1.4/1 in NaH_2PO_4, KH_2PO_4 (I), $Ca(H_2PO_4)_2$ and $CaHPO_4$. In the KH_2PO_4 (II)diet, PM and BM, dietary Ca/P was 1.9/1.Each diet was fed for 13 days of adaptation, then a 5-day balance trial was conducted, followed by a ≥14 day washout period. On day 5 of the balance trial, blood was sampled at 8 different time points and area under the curve (AUC; mmol/l*t) was calculated for serum P. In the present study, the experimental data was analysed as follows: faecal Ca (x-axis) was plotted against faecal P (y-axis; both in mg/kg body weight$^{0.75}$) and the regression lines were compared with the test of Ho. The difference between dietary Ca/P ratio and faecal Ca/P ratio was calculated (Δ) and compared between diets (Holm-Sidak all pairwise test). Δ was correlated to AUC(P).

Results: In P_o diets, faecal Ca and P showed a strong linear correlation ($y = 0.47x – 22.24$; $R^2 = 0.99$), while in P_i diets, the correlation was weak ($y = 0.13x + 182.91$; $R^2 = 0.15$). The regression lines differed significantly ($p<0.01$). The P_o sources PM, BM <1mm and BM >1mm did not differ significantly in Δ (means±SD: -0.27±0.09, -0.33±0.12 and -0.20±0.05, respectively). They had significantly higher Δ values than all other diets ($p<0.001$). There was no significant difference between Δ of NaH_2PO_4 and KH_2PO_4 at dietary Ca/P ratios of 1.4/1 and 1.9/1 (-1.41±0.15, -1.31±0.24 and -1.40±0.25, respectively). Between Δ and AUC(P), a significant inverse correlation ($y = -7.94x + 7.60$; $R^2 = 0.74$) was observed.

Discussion: P_i and especially KH_2PO_4 disrupts the otherwise remarkably strong linear correlation between faecal Ca and faecal P that was also shown in a previous study on different species (3). Relative to faecal Ca, less P was excreted via faeces in the P_i diets, hinting at a higher availability of P_i sources. Absorption of highly available P_i might occur before insoluble Ca-P-complexes (3) can be formed in the intestinal lumen. Extremely low Δ values were found in the P_i diets, especially in Δ of KH_2PO_4 and NaH_2PO_4, which also indicates relatively higher P availability. The diets with $Ca(H_2PO_4)_2$ and $CaHPO_4$ had significantly higher Δ values than the sodium- and potassium-phosphates. The inverse correlation between Δ and AUC(P) indicates that Δ may serve as a predictor for serum P elevation, unlike aD(P) (2).

Conclusions: Balance data used in this study confirms differences in intestinal Ca and P handling dependent on P source. P_i sources seem to have a higher availability, resulting in lower Δ values than those of P_o sources. Therefore, Δ could serve as an easy to determine parameter to help distinguish P sources in pet food. The dietary Ca/P ratio did not influence Δ in KH_2PO_4 diets.

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* *Ludwig-Maximilians-Universität München Chair of Animal Nutrition and Dietetics, Schönleutner Str. 8, 85764 Oberschleißheim, Germany, linda.boeswald@lmu.de*

45.

Comparison of ionized and total calcium level in whole blood and serum, respectively, of multiparous cows around parturition

Vergleich zwischen ionisiertem und Gesamtcalciumgehalt von Vollblut bzw. Blutserum bei multiparen Kühen im Geburtszeitraum

*Ott D., Rosendahl J., Schrapers K. T., Aschenbach J. R. – Berlin

Around calving, the demand for calcium increases dramatically due to colostrum and milk production. This demand cannot be covered from the blood calcium pool alone and needs to be compensated by calcium entry from the bone or gastrointestinal absorption. During this period, the animals are prone to develop subclinical hypocalcemia or even milk fever. Multiparous cows have an incidence of 30-60% to suffer from subclinical hypocalcemia, which increases their risk for secondary diseases like displaced abomasum or metritis [1,2]. Usually cows are classified to be subclinically hypocalcemic if serum calcium level falls below 2.0 mmol/L. Ionized calcium is mostly not determined, even though it represents the biologically relevant and available form. It was estimated that 50% of total calcium is not bound to proteins or anions and thus present in the ionized form. The aim of the current study was to compare total and ionized calcium levels in blood serum and whole blood, respectively, around parturition and to determine the correlations between these two parameters.

Methods: Blood samples were taken from 14 multiparous, Holstein Frisian cows at days -2, 0, 2, 4, 7 and 14 (± 1) relative to parturition. Blood obtained from the vena coccygea was filled either into heparinized vacutainers for the determination of ionized calcium (iCa) or into serum vacutainers for the analysis of total calcium (tCa) from the serum. ICa was immediately measured using an ion selective electrode (Stat Profil Prime, Nova Biomedical), either directly from the vacutainer (iCa_Vac) or from a heparinized blood capillary (iCa_Cap). Serum samples were frozen until wet chemical analysis of tCa (Indiko Plus, ThermoFischer Scientific).

Results: The correlation coefficients between iCa_Vac to tCa and iCa_Cap to tCa were 0.59 and 0.43. Linear regressions were estimated as iCa_Vac = 0.13 * tCa + 0.82 mmol/L ($r^2 = 0.35$; $P < 0.01$) and iCa_Cap = 0.11 * tCa + 0.76 mmol/L ($r^2 = 0.18$; $P < 0.01$). Although both correlations were statistically significant, the high intercepts clearly identified a problem in the assumption that 50% of tCa is freely available. The values for iCa_Vac and iCa_Cap showed a higher correlation ($r = 0.91$) with a linear regression of iCa_Ca = 1.04 * iCa_Vac - 0.18 mmol/L ($r^2 = 0.826$; $P < 0.01$). These parameters correlated much better, but the further transfer of blood sample into the heparinized capillary lowered the iCa by ~0.18 mmol/l. We further compared the relative changes of iCa and tCa around calving by calibrating all calcium data to the respective value at d-2 (= 100%) for each individual cow. Two-way ANOVA with post-hoc Tukey test revealed that all relative calcium levels decreased from day -2 to the day of calving (to 79 ± 4% for tCa and 88 ± 3% for iCa_Vac, $P < 0.05$) and recovered to basal values very fast ($P > 0.05$ for d2, d4, d7, d14). The relative levels of tCa decreased sharper and recovered slower, resulting in differences between methods: At the day of calving, relative tCa values were significantly lower compared to relative values of iCa_Vac and iCa_Cap ($P < 0.05$). At day 4, relative values for tCa was lower than for iCa_Cap ($P < 0.05$) but not different from that of iCa_Vac. The relatively stronger decrease in total calcium reveals a shift in the ratio of tCa/iCa directly at calving, suggesting that more calcium is freely available than one would expect regarding tCa results.

Conclusions: Our data reinforce the need to measure the biologically relevant ionized calcium. An extrapolation from tCa to iCa does not provide accurate information around calving. The heparinization of the sample vessels must be taken into account when evaluating iCa values and double heparinization should be avoided. Although the determination of iCa is technically more demanding due to the needs for immediate measurement without exposure to air, its implementation into practice is urgently required for adequate assessment of the calcium status. A bigger data base on iCa dynamics around calving is necessary to better understand the development of hypocalcemia and milk fever.

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* *PerformaNat GmbH, Hohentwielsteig 6, 14163 Berlin, Germany, dorothee_ott@web.de*

46.

Effects of copper sources and doses on growth performance and oxidative status of weaning piglets

Wirkung von Kupferquellen und -dosen auf die Wachstumsleistung und den oxidativen Status von abgesetzten Ferkeln

Rodriguez M., *Monteiro A. N., Romeo A., Montalvo G., Morales J. – Segovia / Annecy

High dosages of copper (Cu) improve growth performance of weaning piglets. However, the new EU Regulation allows a maximum Cu content in the diet of 150 ppm Cu until to 4 weeks post-weaning (PW) and 100 ppm from 5 to 8 weeks PW. As these levels are still higher than nutritional requirements, further reductions might be possible but with risk of losing growth promoting effects.

Methods: Three hundred eighty-four piglets, divided in 6 groups (8 pens per group, 8 piglets per pen) were used. During 5 weeks, piglets were fed with Cu sulphate ($CuSO_4$) or dicopper oxide (Cu_2O; CoRouge®, Animine) at different dosages leading to total dietary content of: 50, 100, 150 ppm of Cu. Performance was measured at the beginning of the trial (weaning) and at 14 and 35 days PW. At the end of the trial, 24 piglets per treatment were slaughtered for the evaluation of oxidative status (malondialdehyde, superoxide dismutase activity) in the liver as well as for microbiota and gut health parameters in the intestine. The experimental unit was the pen for the zootechnical performance and the piglet for the measurements in the liver and in the intestine.

Results: Piglets fed 150 ppm Cu from Cu_2O had higher final body weight (19.8 kg) than those fed $CuSO_4$ (17.9; $P<0.05$) with a numerical improvement of the feed conversion ratio ($P>0.10$) from 15-35 days (1.44 *vs.* 1.50). Hepatic Cu did not change significantly ($P>0.05$) according to treatments (mean values between 12 and 20 mg/g), and it can be due to the lack of difference on feed intake (average of 455 g/d). Superoxide dismutase (SOD) activity was affected by Cu dosages ($P<0.05$), when 150 ppm seems to inhibit SOD activity (5784 U/g) compared to 100 and 50 ppm (mean value of 6202 U/g). At 150 ppm, the group which received $CuSO_4$ had 46% higher malondialdehyde concentration than the group Cu_2O.

Conclusions: The results indicate that the response to copper supplementation in weaned piglets may depend on the source. Some changes were observed in oxidative status, but these differences cannot fully explain the differences in growth performance. Complementary analyses, including microbiota and the expression of genes related to gut health, are in progress.

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* *ANIMINE, Annecy, France, amonteiro@animine.eu*

47.

Efficacy of high doses of phytase on apparent ileal nutrient digestibility, intestinal histology and tibia mineralization in broiler chickens from 1 to 21 days of age

Wirksamkeit hoher Dosen von Phytase auf die scheinbare ileale Nährstoffverdaulichkeit, Darmhistologie und die Knochenmineralisierung bei Masthühnern im Alter von 1 bis 21 Tagen

*Zentek J., Martínez-Vallespín B., Ader P., Männer K. – Berlin / Lampertheim

Phytic acid has a negative effect on phosphorus and nutrient digestibility and might directly or indirectly affect the intestinal mucosa (1). The aim of the experiment was to show whether these effects would be ameliorated by phytase supplemented to a basal diet rich in phytic acid fed to broiler chickens from d 1 to d 21 of age (21-d feeding period) using dose levels of 500 FTU, 1,000 FT, but also 3,000 FTU/kg feed, respectively, a dose level far beyond the average used in practice.

Methods: From 1 to 21 days of age, broiler chickens were assigned to five dietary treatments (T1-T5): A "phytate-free" diet based on Hermetia meal and corn starch (T1), and phytate-rich diets with corn, soybean meal and sunflower meal (T2-T5). The phytate-rich diets were prepared without (T2) and with supplementation of Natuphos® E at levels of 500, 1,000 and 3,000 FTU /kg (T3-T5). For each treatment, 14 replicates with 2 broilers/cage (♂) were used.

The diets were formulated to meet or exceed the nutrient requirements for broiler chickens recommended by GfE (1999) and contained per kg 12.4 MJ ME, 224 - 231 g crude protein, 8.6 – 9.2 g calcium and 7.5 (T1) or 4.4 – 4.6 g phosphorus (T2-T5). Performance was measured on a weekly basis, the apparent ileal digestibility of ME, phosphorus, nitrogen and amino acids was measurements at study end using titanium oxide as indigestible marker. The left tibia was freeze-dried, pooled and defatted (2 birds each) for measuring crude ash, phosphorus, and calcium. Histo-morphometry was performed in the jejunum and caecum using formalin fixed tissues. Statistical analysis was performed with SPSS, using ANOVA and Tukey test ($P < 0.05$).

Results: Broiler chickens fed the T1 diet reached a lower body weight compared to all other groups. Birds consuming the phytate-rich corn-soybean meal-sunflower meal diet showed a higher performance with an increase in the level of phytase. Increasing phytase inclusion to the phytate-containing basal diet resulted in improvements of apparent ileal digestibility of crude protein ($p< 0.001$) from 72.1 (T2) to 80.4 % (T4) and of all measured amino acids, but the effect was only seen up to dosage of 1,000 FTU/kg feed. The highest relative apparent ileal phosphorus digestibility was observed with the phytate free diet (65.5 %), phytase supplementation resulted in positive responses of relative apparent ileal digestibility of phosphorus ($p<0.001$) from 37.5 (T2) to 41.1 (T3), 54.6 (T4) and 60.1 % (T5), respectively, while no effect was observed for calcium. Birds fed the phytate containing diet T2 had reduced tibia ash (-17.3 %) and phosphorus (-26.7 %) compared to T1. Increasing phytase resulted in a dose dependent improvement of ash, phosphorus and calcium contents up to 31.3, 63.3% and 46.0% ($p<0.001$). The results of histomorphological measurements in the jejunum and in the caecum did not result in significant differences between the treatments in both localizations. When the data are put in relation to body mass or metabolic body mass, relatively clear differences were observed, which are particularly noticeable when comparing T1 with the other treatments but also T2 with phytase supplemented groups.

Conclusions: The studies underline that diets with high phytic acid content have negative effects on performance, ileal digestibility of nutrients and mineralization of the tibia. An increase in the phytase dosage led to a significant improvement in these parameters compared to the standard recommendation. The observed impact of phytase on gut histomorphology requires further validation. The lower performance in birds from group T1 is probably related to a reduced palatability of the diet.

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* *Freie Universität Berlin Institute of Animal Nutrition, Königin-Luise-Str. 49, 10439 Berlin, Germany, juergen.zentek@fu-berlin.de*

48.

Trace mineral contents in different sections of the mane and tale hair in horses

Der Gehalt an Spurenelementen in verschiedenen Abschnitten des Mähnen- und Schweifhaars von Pferden

*Saliu E. - M., Zentek J. – Berlin

Hair analyses are commonly used to evaluate the trace mineral status in horses. In the literature, the analyses generally relate to the entire hair and the reported trace mineral contents vary considerably. It was the aim of this study to compare different longitudinal sections of mane and tale hair regarding the content of copper, manganese, iron and zinc.

Methods: The full length of mane and tale hair was collected from 11 healthy horses aged 6 to 33 years (hair colour: 8 black, 2 white and 1 brown) using pottery scissors. After extensive washing and rinsing, the mane was cut into 3 pieces of 5 cm length each, while the tail was divided into 4 sections of each 20 cm. The division was performed starting from the anterior part and the last section was not cut to a defined length but contained the entire ending. The hair was dried overnight at 103 °C, crude ash was determined and the contents (mg/kg DM) of copper, zinc, manganese and iron in the hair were analysed using atomic absorption spectroscopy (contrAA 700, Analytic Jena AG, Germany). Also, the relative content of trace element per crude ash was calculated. Statistical analysis were performed with IBM SPSS (Version 22). The non-parametric Kruskal-Wallis test was used to determine significance and subgroups. Differences were considered statistically significant if the probability of $P < 0.05$.

Results: Crude ash and the content of copper, iron and manganese differed significantly between sections of mane and tail hair in horses (4.4 – 13.0 g/kg DM, 6.9 – 11.2 mg/kg DM, 15.0 – 68.8 mg/kg DM and 1.3 - 15.2 mg/kg DM respectively), while the zinc content was rather constant (134.9 – 227.3 mg/kg DM). In tail hair, compared to the anterior part, the posterior part showed 2.5-, 1.3-, 8.9- and 4.6-fold increases of crude ash, copper, manganese and iron respectively. Also, the relative abundance of copper (0.8 – 1.9 mg/g crude ash) and zinc (14.1 – 40.5 mg/g crude ash) per crude ash content was significantly different between sections. A 2.3- and 2.7-fold reduction was observed comparing the relative copper and zinc contents in posterior sections to the anterior parts of tail hair respectively.

Conclusions: It can be concluded that the section chosen to evaluate the trace mineral content in mane and tail hair in horses has a significant impact on the results and should be reported.

** Freie Universität Berlin Institute of Animal Nutrition, Königin-Luise-Str 49, 14195 Berlin, Germany, eva-maria.saliu@fu-berlin.de*

49.

Effect of two different weaning ages and mothers' parity on glucose metabolism and growth of female Holstein calves

Einfluss unterschiedlicher Absetzalter und Parität der Mutter auf den Glucosestoffwechsel und das Wachstum weiblicher Holstein-Kälber

*Schwarzkopf S., Kinoshita A., Huber K., Dänicke S., Frahm J. – Stuttgart / Braunschweig

Primiparous cows still grow and develop during their first pregnancy and have to allocate resources between their own ongoing growth and the calf's development. Consequential potential restriction of the calf can have a major impact. Weaning can be a stressful event that causes a rapid development from pseudomonogastric to a full ruminant. For this, adequate maturity of various organs and tissues is needed. Hypothetically, a higher weaning age may cause a more mature status. This may lead to a smoother transition of the energy metabolism to the ruminant status and better growth around weaning.

Methods: The study aimed to evaluate effects of weaning age and mothers' parity on fifty-nine female Holstein calves (38.8 ± 5.3 kg of birth weight, 8 ± 2 days old). They were randomly divided into 4 groups: calves born to primiparous cows, weaned at week 7 (earlyPC; n = 15), calves born to primiparous cows, weaned at week 17 (latePC; n = 12), calves born to multiparous cows, weaned at week 7 (earlyMC; n = 16), and calves born to multiparous cows, weaned at week 17 (lateMC; n = 16). Blood samples were taken on experimental day 1, 28, 42, 70, 98, 112 and 140. Serum glucose concentrations were determined by using an automatic analyzing system, based on spectrometric methods (Eurolyser, Type VET CCA, Salzburg, Austria). Plasma insulin concentration was analyzed using a bovine insulin ELISA (Mercodia, Sweden). To evaluate growth, live weight and morphometric parameter (hip height, back length and heart girth) were measured at day 1, 28, 42, 70, 98, 112 and 140. Morphometric measures was additionally recorded at day 7, 14, 56, 84 and 126. Results were presented as least squares means (LSMeans) and standard errors (SE) which were evaluated by repeated measures using PROC MIXED procedure in SAS (V 9.4., SAS Institute Inc., Cary, NC, USA). The model included fixed factor of time, weaning age, parity of the mother and their interactions while the time was taken into consideration by a "REPEATED" statement. Significant effects were further tested with Tukey-Kramer procedure using procedure PDIFF. For all statistical tests $p < 0.05$ was the level of significance.

Results: Interactions between time and weaning group were observed to be highly significant for live weight, hip height ($p < 0.001$) and heart girth ($p < 0.01$). Live weight was greater for all calves in the late-weaned group from day 70 until the end of trial ($p < 0.01$). Hip height was also significantly greater for late-weaned calves after day 56 until day 84 ($p < 0.05$) with no differences between PC and MC. Later weaned calves had a greater heart girth from day 84 on ($p < 0.05$). Back length was the only morphometric variable which was influenced by parity and weaning age in an interactive manner ($p < 0.05$). It was significantly lower for earlyPC compared to earlyMC ($p < 0.01$). Glucose and insulin also showed an interaction of time and weaning age ($p < 0.001$). Serum glucose concentration declined during and after weaning in the early-weaned group. Thereafter, glucose concentrations increased significantly from day 70 until day 140 in early-weaned groups ($p < 0.001$). Insulin concentration decreased with weaning in the early-weaned calves and stayed below insulin concentrations of late-weaned calves on day 98 and 112, whereas late-weaned calves did not experience such a steep decline in insulin concentration during their weaning.

Conclusions: A weaning age of 17 weeks enabled smooth transition into ruminant status. As mothers' parity affected the back length of early-weaned calves, its potential influence on health and development should be considered in further research.

* *University of Hohenheim Department of Functional Anatomy of Livestock, Fruwirthstraße 35, 70593 Stuttgart, Germany, sarah.schwarzkopf@uni-hohenheim.de*

50.

Clustering of *ad libitum* milk-fed dairy calves according to milk feeding behaviour and its impact on feed intake, growth performance and metabolic and endocrine traits in blood

Clusterbildung bei ad libitum gefütterten Milchkälbern mit Bezug auf das Tränkeverhalten und dessen Auswirkung auf Futteraufnahme, Wachstum und metabolische und endokrine Blutparameter

Ghaffari M. H., Hammon H. M., Frieten D., Gerbert C., Dusel G., *Koch C. – Bonn / Dummerstorf / Westerau / Münchweiler an der Alsenz / Bingen

Dairy calves are able to digest and metabolize large amounts of milk when liquid feed is freely available (1). However, *ad libitum* milk replacer (MR)-fed calves largely variate in their feeding behaviour and individual feed intake during the preweaning period (2). The aim of the present study was to detect possible similarities between milk feeding behaviour in specific subgroups of calves with free access to MR and to explore how it can affect feed intake, growth performance and blood metabolites and hormones. The study intends to classify *ad libitum*-fed calves according to their individual voluntary MR intake using a clustering approach.

Methods: Holstein calves (16 male and 16 female) were studied from birth until d 80 (±2). All calves received first colostrum (2.5 kg) from their dams within 2 h after birth. Subsequent colostrum meals and MR (125 g powder/l) were offered *ad libitum* for 8 wk. Half of the calves received a MR with 0.33% Ca-/Na-butyrate (1). Milk intake was stepped down to 2 l/d from wk 9 to wk 10, and 2 l MR were offered until the end of the study. Concentrate, hay and water were freely available. Calves were housed in straw-bedded group pens with automatic feeders for MR and concentrate intake, where feed intake and feeding behaviour were documented (3). On d 1, 2, 4 and 7, then weekly until wk 11 of life, blood samples were taken to measure plasma concentrations of glucose, β-hydroxybutyrate (BHB), total protein, urea, insulin, insulin-like growth factor (IGF)-I and glucagon. The K-means cluster procedure of the SAS package (PROC FASTCLUS; SAS Institute Inc., Cary, NC) was used to generate clusters. The mean, sum, and standard deviation of MR intake/rewarded visit during the entire study were included as features for the clustering process to classify the calves into number of groups. The R-squared for predicting the variable from the clustering in this model was 0.94. After identifying cluster groups, we analysed the data in SAS using PROC MIXED with repeated measures for time point comparison. The model consisted of sex, meal size, butyrate supplementation, time and respective interactions as fixed effects, and calves as a random effect. The threshold of significance was set at $P \leq 0.05$; trends were declared at $0.05 < P \leq 0.1$.

Results: Two groups of calves were identified as high MR intake/rewarded visit (HIGH; 2.2 ± 0.11 l MR/visit; n = 12; 7 male, 5 female) and low MR intake/rewarded visit (LOW; 1.8 ± 0.07 l MR/visit; n = 14; 4 male, 10 female). The rest of the calves were not included in the statistical analysis. Butyrate supplementation was equally distributed within clusters and did not affect MR meal size. Dry matter intake (DMI) of MR did not differ between HIGH and LOW, but DMI of concentrate and total DMI tended to be greater (P=0.1) in HIGH than in LOW and increased (P<0.01) at the end of the study more distinct in HIGH than in LOW (P<0.01). Average daily gain was greater (P<0.05) in HIGH than LOW and was reduced (P<0.05) by butyrate feeding. Body weight increased lowest in LOW calves with butyrate intake. Plasma concentrations of total protein, glucose, urea, insulin, and glucagon were higher (P<0.05) and concentrations of IGF-I tended to be higher (P<0.1) in HIGH than in LOW. Plasma BHB was higher (P<0.05) in LOW than in HIGH at d 63 and lower in calves fed MR with butyrate (P<0.05) at d 77. Plasma IGF-I concentration was higher (P<0.05) in calves fed MR without butyrate than with butyrate.

Conclusions: Significant clustering for meal size emphasize the individual variation of MR intake in *ad libitum* fed calves. A greater concentrate intake at the end of the study in HIGH calves indicates that the capacity for feed intake referred to both, MR and solid feed intake. Parameters in blood plasma supported the stimulation of body growth in calves with a greater milk meal size per rewarded visit.

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* *Educational and Research Centre for Animal Husbandry, Münchweiler an der Alsenz, Germany, c.koch@neumuehle.bv-pfalz.de*

51.

How to improve the early supply of newborn piglets at increasing litter size – test on a new automatized liquid feeding system for suckling piglets

Wie ist die Versorgung neugeborener Ferkel bei steigender Wurfgröße zu sichern? Feldstudie zum Einsatz einer neuen automatisierten Flüssigfütterungstechnik für Saugferkel

*Paß S., Keller B., Tenhündfeld J., Rohn K., Kamphues J. – Hanover / Vreden

In pig production there was in the last decade a tremendous increase in litter size. The number of piglets often exceeds the number of productive teats. Thus specific efforts are required to ensure a high survival rate. Aim of the present field study was to test a new automatized liquid feeding system for suckling piglets – in comparison to a manual offer – regarding the intake of the liquid diet, losses and performance of the differently supplied litters/ piglets and potential side effects on the sows.

Methods: On one farm specialized on piglet production with 500 sows (Danish line) of high reproductive performance two different forms of feeding the suckling piglets were compared. A modern automatized supplementary feeding technique (PigStart, Tewe Electronic, Vreden) for offering the feed as a liquid diet in a high frequency was compared with a traditional offer by hand (2x/d). Feeding a liquid diet started at the d2, in the first 5 days a liquid milk replacer diet was offered, beginning at d6-7 a prestarter was used up to d17, already at d18 a normal compound feed for reared piglets was fed in a liquid form (12/16/20 % DM in the offered liquid diet). The diets mixed in the new equipment were also used for offering manually, i.e. identical diets were used in both types of feeding the piglets. Weight at birth and gains, loss rates, intake of supplementary feeds, and clinically obvious problems were monitored during the entire suckling period (up to d24). Furthermore the feed intake of sows, their bw and backfat thickness at parturition as well as at rearing were measured. Data were statistically analysed using the SAS® Enterprise Guide® (version 7.1, Fa. SAS Institute Inc. Cary, NC, USA). The normal distributed values (bw, piglets/ litter) were analysed by using the one or the two-way Anova test. For not normally distributed values (feed intake) the two-samples Wilcoxon test was used.

Results: In total data on 86 sows (bw after parturition: Ø265 kg) and 94 litters were available, 40 litters were supplied by hand (twice a day), the others were fed by the new sensor directed automatized liquid feeding technique (> 6 meals a day). The new technique was well accepted by the piglets already at d3/d4; filling the trough with the liquid diet was accompanied by a noise signal with attractive effects on the piglets. The number of piglets born alive was 17.5±3.53 in the group with the automatized feeding technique and 17.7±3.8 in the group supplied by hand. At these litter sizes the birth weight varied at 1.24±0.33 kg in both groups. The total intake of DM from the supplementary feed (offered as liquid diets) reached values of 551g per one piglet during the entire suckling period when the diet was offered by the automatized technique and 297 g when the liquid diet was offered manually. Compared to the feed supply by hand (13.0 reared piglets/litter) the modern feeding automatized technique resulted in an increased litter size at rearing (13.5). The average bw at rearing was not effected by the kind of the supplementary feeding 6.45±1.55 kg (automatized feeding technique) vs. 6.40±1.42 kg (supplied by hand). The intensified creep feeding did not result in a marked reduction of losses during the suckling period. Furthermore, it is noteworthy that the gains` variation of individuals within the litters was not lowered as it was intended. No statistical differences could be identified for these results. Differences were observed between avg. daily feed intake of the automatically-supplied and hand-fed piglets, especially towards the end of the suckling period ($p<0.05$).

Conclusions: The advantages of the tested automatized feeding technique compared to manual feeding are obvious, especially regarding to the work / time required for a more frequent feeding. This modern feeding technique has further positive effects at rearing, the piglets are used to the liquid diet that is also offered after rearing what helps to avoid a starvation for one or two days after rearing. Finally, this technique demonstrated the efforts of the farmer regarding the fate of newborns, which do not have their individual/own teat, i.e. exposed to thirst and hunger and early death.

* *University of Veterinary Medicine Hannover, Foundation Institute for Animal Nutrition, Bischofsholer Damm 15, 30173 Hanover, Germany, s.pass@gmx.de*

52.

Organic turkey fattening: Required riboflavin supply in rearing of heavy turkey genetics

Ökologische Putenmast: Bedarfsgerechte Riboflavinversorgung in der Aufzucht schwerer Putenherkünfte

*Göppel S., Weindl P. A., Pleger L., Bellof G. – Freising

The present study should clarify the following questions: What is the vitamin B_2 requirement for organic turkey rearing in contrast to existing references for conventional turkey fattening? Is it possible to reduce the vitamin B_2 supply in case of reducing the supply of essential amino acids? How do different amounts of amino acids and various riboflavin (= vitamin B_2) levels in complete diets affect the fattening performance of heavy bred turkeys "B.U.T. 6" in the rearing phase?

Methods: A 3-factorial test design with 2 different levels of amino acid supply within 6 different levels of riboflavin supply and 2 different locations was conducted. The experiment was synchronously carried out at the testing stations in Kitzingen and in Weihenstephan under organic feeding regime. The investigations focused on roosters of the heavy and fast growing genotype "B.U.T. 6" in the rearing (phase 1: 1st to 28th day). The 576 male poults were divided into 12 feeding groups (4 replicates each; n=12 per pen). The parameters feed consumption, body weight gain, feed conversion rate and animal losses have been considered. In addition, studies on the motor skills of the animals as well as organ and tissue examinations were carried out. By examining the sciatic nerve, a riboflavin deficiency can be proven or rejected. The energy content (AME_N) of the diets was reduced by about 10 %, in contrast to conventional recommendations of the breeding company "Aviagen" (Cheshire, UK) [2]. AME_N levels of 11 MJ ME in the feed mixtures were aspired. According to this reduction, the contents of the amino acids in the mixtures were also reduced for the first limiting amino acid methionine (group 1: 5.8 g/kg and for group 2: 5.1 g/kg complete diet). In the feed mixtures, the supply of vitamin B_2 was reduced in 10 % steps to a level of 50% of the recommendations for both amino acid levels. Therefore, 6 riboflavin levels ranging from 8.9 mg to 4.0 mg vitamin B_2 per kg diet were tested (groups 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6). When calculating the experimental diets, the native vitamin B_2 content of the raw materials was taken into account. The supplementation was made with the organic certified riboflavin product "EcoVit R" from the company "Agrano" (Riegel, Germany).

Results: During the feeding trial, the number of animal losses was low (3.3 %), which indicates an undisturbed test procedure. Losses occured primarily in the feeding groups with the lowest riboflavin supply (groups 1.6 and 2.6). At the end of phase 1 animals fed with the lowest content (x.6) of riboflavin showed significantly the lowest results in feed consumption, weight gain and worse motor skills. In the other riboflavin levels, no significant differences were found. Group 1 with the higher amino acid content showed higher results in the parameters feed consumption, weight gain and had a lower mortality rate than group 2. The pathological examination confirmed at least slight neuropathy on the sciatic nerve in 10 out of 12 examined animals (groups 1.6 and 2.6). This is clearly attributable to a riboflavin deficiency.

Conclusions: According to the recommendation of the GfE [1], but less than recommended by Aviagen, a content of 4 mg of riboflavin per kg of complete diet should be obtained from organically reared livestock turkeys of heavy origin. The low feed capacity of turkeys in rearing does not make it possible to compensate a lower content of amino acids and riboflavin in the diet by increasing feed intake.

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* *Hochschule Weihenstephan-Triesdorf, Am Staudengarten 1, 85354 Freising, Germany, sina.goeppel@hswt.de*

53.

Precision livestock farming technology for weaned piglets

„Precision livestock farming"-Technologie beim Absetzferkel

Papadopoulou E., Moser L., Waxenecker F., Gruber C., *Wein S. – Getzersdorf

The Food and Agriculture Organization of the United Nations (FAO) expects a globally increasing demand for food and feed by 70% in the first half of this century. Crops, used for industrial purposes, will parallel this demand. At the same time, there are concerns about animal health [1] (e.g. transmissionable diseases, use of antibiotics) and animal welfare [2]. While farmers face a decent economic return, they are expected to manage a wide range of processes including animal health as well as animal welfare, product quality, biosecurity, and reduction of greenhouse gas emissions. These requirements can only be accomplished by using new approaches, such as continuous automated real-time monitoring of animal behavior and health. The AsservaSystem *PigInsight* is unique for automated feeding and weighing of small piglets at weaning age. RFID (radio-frequency identification) ear chips permit individual recognition of each animal. The system allows for individual monitoring of animals' feed consumption and thus helps to control the feed quantity during a day. Additionally, animals are automatically weighed whenever entering the drinking station. The aim of this study was to compare and test daily automated weighing with manual weighing.
Methods: Seventy-six healthy piglets (mixed sex; four weeks old, Austrian genotype Ö-HYB-F1 [(Landrace x Large White) x Pietrain]) were allocated to 6 slatted floor pens with balanced mean body weight, sex ratio, age and relatedness among the groups for from day 1 to day 49 after weaning. All animals were fitted with RFID ear-tags. Feed (*ad libitum*, 12 g per demand) and water (*ad libitum*) were provided through two Asserva feeders and drinkers per pen. Climate conditions were computer-regulated according to standard recommendations for weaning piglets and recorded daily. In addition to automated daily monitoring, individual body weight was measured manually on days 1, 7, 21, 28, 35 and 49.
Results: Data derived by daily automated voluntary weighing quantifies instantaneous the animals' weight without causing any stress and extra labour. During our verification period a mean deviation of 0.2633 kg +/- SE 0.1609 (n= 6 weighing occasions) between manual and automated weighing was recorded.
Conclusions: Statistical methods cannot answer the question how far apart measurements can be without leading to problems, but is rather a question of clinical judgment [3]. While automated measurments provide a value derived from plenty of weighings during 24 hours, manual weighings are only a spot sample. The observed deviations in manual and automated weighing can be attributed to differences in feed / water intake and defacation during the day. Automated measurements, however, are considered superior because important parameters such as the average daily weight gain or average daily feed intake are true measures and are not calculated values between manual weighing or feeding intervals.

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* *Biomin Holding GmbH Research & Development, Erber Campus 1, 3131 Getzersdorf, Austria, silvia.wein@biomin.net*

54.

Feeding and diet as influencing factors for the occurrence of tail lesions in weaner pigs

Fütterung und Rationsgestaltung als Einflussfaktoren für das Auftreten von Schwanzveränderungen bei Absetzferkeln

*Grümpel-Schlüter A., Dippel S. – Braunschweig / Celle

Tail biting is a major problem in German pig industry, which can affect pigs of all ages and in every housing system. Especially in undocked pigs, tail biting often occurs in the rearing phase, but also in docked pigs tail biting is a problem during rearing. Tail biting is influenced by many different factors. Besides factors like housing and management (1), the feeding system and the diet composition influence the occurrence of tail biting (2). Because feeding and diet in turn include various factors, the aim of the study was to find risk factors out of these categories, that influence the occurrence of tail lesions in weaner pigs.

Methods: Data were collected on 25 farms throughout Germany, which were visited up to three times with a period of six months between visits. During visits, data on various influencing factors were collected. Additionally, pigs were assessed due to lesions on tails. Overall 368 pens were assessed with in median 25 pigs per pen. For the identification of risk factors, a regression tree analysis (RT) was done using R 3.4. Since in this study feeding and diet are focused, only variables out of these categories were used in the RT. Seven factors were included in the RT: crude fibre content, crude protein content, number of feeding phases during rearing, feeding system, feed analysis during last 12 months, structure of feed and blending of feeding phases over a period of at least five days. The dependent variable used for RT was the prevalence of tail lesions on pen level. To prune the regression tree a fivefold cross validation was done and additionally, it was defined that in each node at least 19 observations (5 % of all observations) must be included. The Spearman correlation between observed and predicted prevalence of tail lesions was calculated to assess the quality of the analysis.

Results: The median prevalence of tail lesions across all pens was 10.0% (minimum: 0.0, maximum: 100.0). The RT identified five main risk factors. If the feed was not blended for at least five days, the prevalence was higher (28.0%, n=101) as if the feed was blended (18.0%, n=267). If the feed was not blended for at least five days, the prevalence was higher if dry feeding (45.0%, n=38) was installed instead of slop feeding (17.0%, n=63). If the feed was blended for at least five days, the prevalence was higher if the crude fibre content was < 3.1 % (35.0%, n=19). If the crude fibre content was ≥ 3.1% (16.0%, n=248), the prevalence was lower. A further factor was the number of feeding phases, whereby the prevalence was lower at > 2 feeding phase (12.0%, n=94) and higher at ≤ 2 feeding phases (19.0%, n=154). At > 2 feed phases the prevalence was lower if the feed structure is flour or meal (6.6%, n=58), and higher if the feed structure is pellets or crumbs (21.0%, n=36). For ≤ 2 feed phases a further splitting was done using again the feeding type. The prevalence was lower if a slop feeding (14.0%, n=75) was installed in pens and higher if a liquid or dry feeding (24.0%, n=79) was installed. The Spearman correlation coefficient between the observed and the predicted tail lesions prevalence was r_S=0.37 ($p < 0.05$).

Conclusions: The identified five risk factors indicate that an adapted feed supply as well as a diet, which meets the nutritional requirements of pigs, can reduce the occurrence of tail lesions. Nevertheless, it should be kept in mind that tail biting is a multifactorial behavioural disorder and therefore other factors must be considered.

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* *Friedrich-Loeffler-Institut Institute of Animal Nutrition, Braunschweig, Germany, angelika.gruempel-schlueter@fli.de*

55.

Weaned piglets tolerate moderate inclusion rates of dietary fibre without deterioration of performance parameters or the barrier function of the gut

Absetzferkel tolerieren eine moderate Menge an faserreichen Futterkomponenten ohne negative Effekte auf die Leistung und die Barrierefunktion des Darms

*Martínez-Vallespín B., Taibo A., Pahnke L., Kochan M., Riedmüller J., Zentek J. – Berlin

It is accepted that a minimum level of dietary fibre (DF) in monogastric diets is needed for a normal digestive function; however, there is not yet a fibre recommendation in the nutrient requirements tables of swine. There are concerns about DF inclusion because it is assumed that it will impair nutrient digestibility and performance parameters. Our hypothesis is that a moderate increase of DF will not negatively affect performance of weaned piglets. This was tested in a feeding trial by including three different by-products from the food industry.

Methods: A total of sixty-four piglets were weaned at 27 ± 1 days of age and randomly distributed into four experimental groups, balancing for gender and body weight. Animals were allocated in pens with two animals each. After a period of adaptation to the new diets of one week, the trial lasted four more weeks. A control diet (CON) was formulated to meet the nutrient requirements of weaning pigs and three more diets were prepared by substituting part of corn and soybean meal with 8% of one of the three fibrous materials to be tested (carrot pomace - CRT; brewers´ spent grain - BSG; carob pods - CRB). The diets were formulated to be isoenergetic and isonitrogenous (13.40 MJ/kg of ME, and 20.78% of CP on average, respectively). The fibre contents of the diets were: CON, NDF=8.96%, ADF=3.80%, TDF=13.27%; CRB, NDF=10.43%, ADF=5.97%, TDF=18.64%; BSG, NDF=11.9%, ADF=5.52%, TDF=15.07%; CRB, NDF=12.6%, ADF=9.41%, TDF=18.09%. Feed intake and body weight were weekly recorded while health status and faecal consistency were daily monitored. At days 35 and 36, eight animals per group were euthanized for sample collection. Liver, empty stomach and caecum were weighted. Small sections of jejunum and middle colon were taken for histomorphological measurements after hematoxylin-eosin staining, and a piece of proximal colon for PCR analyses to quantify expression of several genes involved in barrier function (MUC2, claudins 1, 2 and 3, occludin and ZO-1). All data were evaluated by ANOVA in SPSS with group as the only independent variable, followed by Tukey post-hoc test.

Results: All the performance parameters measured (body weight, daily weight gain, daily feed intake and feed conversion rate) showed no effect of fibre sources. Likewise, caecum and liver weight relative to body weight were not affected by any of the tested ingredients. However, animals fed the CRT diets had a slightly lower stomach weight (P=0.032). Histological measurements (jejunum villus length and crypt depth and colon crypt depth) showed no significant differences. Expression of the selected genes related with barrier function was similar in all the groups. Finally, faecal score during the end of second week and most of the third week pointed out the animals in groups BSG and CRB having firmer faeces.

Conclusions: The results of the study showed that a moderate increase of 1.5% to 3.6% of NDF in the diets of recently weaned piglets, reaching values up to 12.6% of NDF, did not affect performance nor histological parameters in the intestine. This would be in agreement with some other previous studies, although contradictory results can be often found, which can be attributed to the highly complex composition of the fibre. Moreover, the higher increase of DF in the diets BSG and CRB (2.9% and 3.6% of NDF, respectively) had a positive effect on the faecal consistency, which might be due to an influence on passage through the digestive tract or water holding capacity.

* *Freie Universität Berlin Institute of Animal Nutrition, Königin-Luise-Str. 49, 14195 Berlin, Germany, beatriz.martinezvallespin@fu-berlin.de*

56.

Effects of substituting soybean meal by rapeseed meal (both: extracted) in rye based diets for young fattening pigs regarding digestibility and performance

Auswirkungen eines Ersatzes von Soja- durch Rapsextraktionsschrot in einem roggenbasierten Mischfutter für junge Mastschweine auf die Verdaulichkeit und Mastleistung

*Wilke V., Grone R., Kamphues J. – Hanover / Bergen

Compound feeds inswine production, especially for growing pigs, currently contain high shares of wheat and soybean meal (SBM) while the levels of rye and rapeseed meal (RSM) are low. However, there are advantages of rye and rapeseed becoming more important. Rye has unique properties regarding tolerance of draught ('climate change') and efficiency of nitrogen and phosphorus utilization. Finally due to progress in rye breeding ('hybrides') there are nowadays improved yields and lower risks for ergot contamination [1]. Rapeseed (extracted meal) is a regional protein source (Germany) and has lower negative impacts on the environment ('CO_2 footprint'), compared to SBM [2]. As already been shown in previous investigations, there are no obvious disadvantages of substituting wheat by rye in diets for young fattening pigs [3]. Therefore, it was of interest to compare groups of young fattening pigs fed a rye based diet (60 % in general) with different shares of RSM to determine the effects of substituting SBM by RSM in a diet on several parameters of performance.

Methods: 2 x 20 pigs [trial 1: age: 47 ± 0.489 days; bodyweight (bw): 15.1 ± 1.57 kg / trial 2: age: 50 ± 0.00 days; bw: 17.8 ± 2.86 kg] were housed individually in four feeding groups of five pigs each. Each group received a diet consisting of rye, SBM and/or RSM, barley, lignocellulose and minor ingredients. The compound feed of each group was characterized by different shares (%) of both protein rich ingredients (SES/RSM: 18.1/0; 13.6/6.70; 8.10/16.1; 0/28.0). Moreover, distinct amino acids were supplemented to ensure comparable amounts in all diets. The animals were fed 4 weeks the respective compound feed variant *ad libitum*. Over the entire experimental period, feed intake (daily) and gains (weekly) were determined individually. In addition, during the second week of the study, the feces were collected completely and individually for 5 days to determine the apparent total tract digestibility at *ad libitum* feeding. One piglet (runt) was excluded from statistics, done by SAS® Enterprise Guide® (Anova; $p < 0.05$).

Results: No negative effects of higher dietary RSM levels regarding feed intake occurred. Furthermore the determined daily weight gains showed no significant differences. During *ad libitum* feeding digestibility rates of organic matter (OM) and crude protein (XP) were lower when SBM was substituted by RSM [digestibility (%) of OM: trial 1: group 1: 85.7 ± 2.29; group 4: 83.7 ± 0.785 / trial 2: group 1: 86.9^{a} ± 0.769; group 4: 84.0^{b} ± 1.08 // digestibility (%) of XP: trial 1: group 1: 78.5 ± 3.99; group 4: 73.9 ± 1.56 / trial 2: group 1: 81.9^{a} ± 1.51; group 4: 73.6^{b} ± 1.64]. Furthermore no negative effects on feces' DM- content were found when shares of RSM increased. In both trials the feed conversion ratio differed significantly when the whole share of SBM was substituted by RSM (trial 1: group 1: 1.58^{a}; group 4: $1,79^{b}$ / trial 2: group 1: 1.62^{a}; group 4: $1,79^{b}$).

Conclusions: Regarding the above mentioned performance parameters (feed intake and gains) there were no obvious disadvantages replacing SBM by RSM. In addition, no negative side effects of RSM regarding feces composition in young pigs were observed. The lower digestibility rates and with that higher values of the feed-conversion-ratio could be compensated (economically) by lower costs of rye based diets supplemented with RSM instead of SBM and distinct amino acids.

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* *University of Veterinary Medicine Hannover, Foundation Institute for Animal Nutrition, Hanover, Germany, volker.wilke@tiho-hannover.de*

57.

Does a high-energy diet and Spirulina supplementation affect body weight of pregnant sows and their piglets?

Beeinflusst eine Hochenergiediät und Spirulina-Supplementierung das Körpergewicht von tragenden Sauen und ihren Ferkeln?

*Lugarà R., Kreuzer M., Giller K. – Lindau

Maternal dietary habits during pregnancy and lactation can influence the offspring with consequences for health and metabolism, even in later life (1). In this respect, a Western diet may have direct and intergenerational detrimental effects by increasing the risk for the development of obesity and related metabolic disorders. The microalga *Arthrospira platensis* (Spirulina, Sp) contains many bioactive compounds (2), which are presumed to mediate metabolic health effects. Although extensive research has been carried out on the beneficial effects of a direct consumption of Spirulina, up to date its potential intergenerational effects are not reported. It should, therefore, be investigated if Spirulina supplementation can ameliorate the negative consequences of a Western diet using pigs as a model organism.

Methods: In the first of two runs, 10 gilts (LandracexLarge White, 5 months old, initial body weight (BW) of 112±6 kg (mean±SD)) were randomly assigned to two dietary groups (n=5). While the control diet (CTR) was based on commercial recommendations for gestating and lactating pigs, the experimental diet (WES) contained high proportions of lipids (15% hydrogenated plant oil), sugars (15% fructose, 20% sucrose) and cholesterol (0.2%). Both diets met amino acid requirements for pregnant and lactating sows. Animals had *ad libitum* access to feed and water. After 2 months of pre-feeding, Sp supplementation (20 g/day) was started for part of each diet group, resulting in four experimental groups, CTR (n=2), CTR+Sp (n=3), WES (n=3), WES+Sp (n=2), and gilts were artificially inseminated with sperm from the same boar. From days 50 to 95 of gestation, feed intake was restricted to 2kg/day to prevent the animals from becoming overly obese. From day 95 to 107, the daily allowance was increased to 3kg and from day 107 onwards *ad libitum* intake was permitted. BW and thickness of subcutaneous adipose tissue and muscle were measured biweekly while energy intake was recorded weekly. Piglet birth weight was determined. Milk yield (kg) was calculated as described in (3). Data were evaluated (IBM SPSS v.26) by ANOVA for repeated measurements followed by Tukey's HSD test for multiple comparisons between combined dietary groups. A Linear Mixed Model was used with diet, Sp supplementation, piglet gender and their interactions as fixed effects and sows as random effect.

Results: Preliminary results from the first exprimental run indicate a higher BW in CTR (266±3kg) compared to WES sows (234±17kg) in week 12 of pregnancy ($p<0.05$). In both diet groups, Sp supplemention tended to lower the BW compared to the respective unsupplemented group (CTR vs CTR+Sp: 235±10 vs 222±9kg ; WES vs WES+Sp: 230±9 vs 205±9kg; $p<0.05$). Subcutaneous adipose tissue thickness did not significantly differ between diets. The WES+Sp sows had a lower muscle thickness than the WES sows ($p<0.05$), but only in week 2 of gestation. The CTR+Sp sows had a lower energy intake than CTR sows (41.3±3.2 vs 46.2±4.0 MJ/day, $p<0.05$) while in the WES+Sp sows it was higher than in the WES sows (54.8±3.8 vs 44.7±3.7 MJ, $p<0.05$). Diets and Sp supplementation did not affect litter size, pregnancy length and daily milk yield. Birth weight of total WES offspring tended to be higher than that of total CTR ($p<0.10$). Sp supplementation in WES sows led to a piglet birth weight comparable to that of CTR piglets (CTR vs CTR+Sp: 1.58±0.19 vs 1.62±0.19kg, WES vs WES+Sp: 1.91±0.15 vs 1.53±0.15kg, $p<0.10$).

Conclusions: The preliminary results of this study indicate that Sp supplementation might reverse the direct and intergenerational effects of a Western diet on animal growth. By increasing the number of experimental animals with the second run, these preliminary results will be extended by increasing statistical power. Potential effects on the epigenetic, transcriptomic and metabolomic status of sows and piglets are currently under investigation.

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* *ETH Zürich Institute of Agricultural Sciences/Animal Nutrition, AgroVet Strickhof, Eschikon 27, 8307 Lindau, Switzerland, rosamaria.lugara@usys.ethz.ch*

58.

Changes of plasma volume and osmolality in diarrheic calves fed with milk, water- or milk-based oral rehydration solutions

Veränderungen des Plasmavolumens und der Osmolalität bei durchfallkranken Kälbern nach Fütterung von Milch, wasser- oder milchbasierten oralen Rehydratationslösungen

Wenge-Dangschat J., Steinhöfel I., Coenen M., Tuchscherer A., Hammon H. M., *Bachmann L. – Leipzig / Köllitsch / Dummerstorf

The administration of oral rehydration solutions (ORS) is an effective method to treat diarrheic calves. ORS can be prepared in milk (milk replacer) or water, respectively. Abomasal passage of milk and milk-based ORS in healthy calves is decelerated compared to ORS prepared in water. The rate of increasing plasma volume is equal in healthy calves when feeding either milk-based or water-based ORS. ORS directly prepared in milk meal are hypertonic. In experimentally dehydrated calves such ORS elevate plasma osmolality, an effect which can antagonize dehydration if water is freely available (1). Under field conditions the administration of ORS via milk meal combined with free water access resulted in higher water consumption in diarrheic calves (2). Further research revealed that abomasal passage is generally delayed in diarrheic calves compared to healthy calves (3). Therefore, the aim of the present study was to elucidate how plasma volume and osmolality change after feeding milk or water- or milk-based ORS to diarrheic calves.

Methods: Experiments started at day 3 ± 1 after the onset of diarrheic disease. After a fasting period of 9 h thirty diarrheic calves (ten per group, age = 11 ± 1 d) were fed either with 2 L of a) milk, b) water-ORS or c) milk-ORS, respectively. Calves were only pretreated with water- or milk-ORS. In every case diarrhea was due to infection with *Cryptosporidium parvum*. Blood samples were taken before and at several time points until 6 h after feeding. Plasma protein (biuret method) and osmolality (freezing point depression) as well as sodium (ion selective electrode) were determined. According to plasma protein, change of plasma volume was calculated. While the feeding experiment calves had free access to water, and water intake was recorded. Data were analyzed by repeated measures ANOVA using the MIXED procedure of SAS System for Windows with diet, time and interaction diet*time as fixed effects.

Results: Plasma volume was raised 30 min after feeding water-ORS ($P < 0.001$) and milk ($P < 0.05$). The rate in increasing plasma volume was not significantly different, but the expansion of plasma volume was less pronounced after feeding milk ($t = 30$ and 120 min, $P < 0.05$) compared to water-ORS ($t = 30$, 45, 60 and 90 min, $P < 0.001$, $t = 120$ and 180 min, $P < 0.01$, $t = 240$ min, $P < 0.05$). After feeding milk-ORS no significant increase of plasma volume could be detected. Plasma sodium increased in calves fed milk-ORS 6 h after feeding ($P < 0.01$) and the increase was different compared to milk-fed calves ($P < 0.05$). Plasma osmolality was higher ($P < 0.05$) at several time points after feeding milk-ORS compared to water-ORS and milk feeding. Despite higher plasma osmolality and sodium in milk-ORS fed calves, water intake was not significantly different between the feeding regimens during the observation period of 6 h.

Conclusions: In calves with diarrhea, plasma volume increase more quickly and to a higher extent after feeding water-based ORS; thus, they should be administered at first when diarrhea occurs. Milk-ORS as hypertonic solutions reveal a higher potential to increase plasma osmolality, in particular sodium; an effect by which thirst is triggered and fluid restoration might take more time but also has the potential to persist longer. As diarrheic calves should be fed with milk to fulfill their energy needs further studies are required to figure out whether a combination of water- and milk-based ORS administration is the most effective way to treat diarrheic calves.

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* *Leibniz-Institut für Nutztierbiologie (FBN) Institut für Ernährungsphysiologie "Oskar Kellner", Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany, bachmann@fbn-dummerstorf.de*

59.

Impact of substituting soybean meal by alternative protein sources in a grass and maize-silage based diet on performance, carcass and meat quality of growing bulls

Einfluss des Austausches von Sojaextraktionsschrot durch alternative Proteinquellen bei einer auf Gras- und Maissilage basierten Fütterung auf Mastleistung, Schlachtkörper- und Fleischqualität von Mastbullen

*Kurrig M., Kreuzer M., Reidy B., Scheurer A., Giller K. – Zurich / Zollikofen

The ongoing discussion about the extensive use of soybean meal (SBM) and the increasing awareness for sustainability of production systems trigger the search for alternative protein sources in livestock nutrition. To ensure a sustained growth performance and carcass quality, alternatives should also cover the ruminant's demand for utilisable crude protein (uCP). Considering the Swiss national programme promoting grassland-based milk and meat production, the aim of this study was to evaluate the effects on performance, carcass and meat quality of growing bulls (1) when fully replacing SBM by faba beans (FB), pumpkin seed cake (PSC), Spirulina (SPI) or no protein source (-P, negative control) while (2) feeding a grass-silage based diet.

Methods: Thirty Limousin crossbred bulls (initial bodyweight: 164±13kg) were used for the study. All bulls were fed a diet composed of grass silage, maize silage and concentrate in a ratio of 0.5:0.3:0.2. Isonitrogenous (~23% crude protein) concentrates contained (g/kg) SBM, 280; FB, 750; PSC, 230; SPI, 200, whereas a grain-based concentrate (14% crude protein) was fed to the –P-group. Individual feed intake, in group housing, was provided by using transponders and electronic doors, and recorded twice weekly. Bulls were weighed every second week. Over a period of seven days, total daily faeces and urine of each animal were collected, weighed and sampled. Individual feed intake was also recorded for calculating the N balance. Bulls were slaughtered at a body weight of about 520 kg. Thus, the experiment comprised 40 weeks. Carcass quality (conformation and fat cover score) was evaluated according to CH-TAX (equivalent to EUROP), and quality of the *L. thoracis et lumborum*, including water holding capacity, Warner-Bratzler shear force (WBSF) and lightness (L*), redness (a*) and yellowness (b*) was measured after 7 and 21 days of ageing. Data was statistically analysed in RStudio (version 1.2.5001) performing an ANOVA (performance) or mixed model analysis (N balance, carcass and meat quality) considering treatment and days of ageing (meat quality) and their interaction as fixed and run as random effects.

Results: Bulls of all treatments showed a similar performance with regards to final body weight, average daily gains (1.43±0.10kg), total dry matter intake and feed conversion ratio. No significant differences were found for faecal N loss and apparent N digestibility. However, the lower N intake of the bulls fed the –P diet, resulted in an about 28% lower urinary N excretion ($p < 0.01$) compared to the bulls fed additional protein. Total N excretion was lower ($p < 0.05$) and body N retention was higher ($p < 0.05$) for bulls fed the –P diet than for the bulls fed FB and SPI. No differences among treatments were found in carcass weight, dressing percentage, conformation score and fat cover score. Regarding meat quality parameters, no treatment differences were found for ageing loss, drip loss, L* a* b* colour values and WBSF, respectively. However, ageing loss, colour values and WBSF were different ($p < 0.05$) for 7 and 21 days aged beef but no interaction (treatmentÍdays of ageing) was found. Cooking losses tended to differ between feeding groups ($p = 0.08$).

Conclusions: These findings show that SBM can be replaced by any of the tested protein sources in a grass-silage based diet without impairing performance or meat quality traits. Bulls fed no additional protein had a comparable performance and meat quality, and a significantly lower urinary N loss. Thus, the results indicate that diets consisting half of grass silage seem to provide adequate uCP to Limousin crossbred bulls, and additional protein supplements only resulted in an increased N excretion potentially harmful to the environment.

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* *ETH Zurich Agricultural Sciences, Universitaetstrasse 2, 8092 Zurich, Switzerland, magdalena.kurrig@usys.ethz.ch*

60.

Long lasting effects of pre-weaning intensive milk feeding: Investigations on T and B cell subsets in blood, thymus, spleen and small intestine of heifers

Langanhaltende Effekte intensiver Milchfütterung vor dem Absetzen: Untersuchungen zu T- und B-Zellverteilungen im Blut, Thymus, Milz und Dünndarm von Färsen

*Liermann W., Viergutz T., Tümmler L.-M., Kuhla B., Hammon H. M. – Dummerstorf

Restrictive milk feeding management in the pre-weaning period is questioned because it inhibits the exploitation of the performance potential of growing calves. Previous studies give evidence that a higher pre-weaning milk supply modulates the jejunal mucosal immune system in calves (1). To study whether there is a long lasting effect of the milk feeding regimen on the immune system the present study focused on the effect of intensive pre-weaning milk replacer (MR) feeding on the adaptive peripheral and local immune system in eight month-old heifers.

Methods: A total of 24 female German Holstein calves were fed either 10% of body weight (BW) colostrum for the first 2.5 d postnatal (p.n.) and thereafter 10% of BW MR, or 12% of BW colostrum for the first 2.5 d p.n. and thereafter 20% MR in the pre-weaning period (n = 12, respectively). In week 9 and 10 p.n. the MR allowance was reduced to 10% MR in all calves. In week 13 p.n. all calves were completely weaned from milk. From week 11 p.n. the calves received a conventional total mixed ratio. Concentrate, hay and water were available *ad libitum* pre-weaning for all calves. In weeks 13-14 the concentrate allowance was reduced to 2 kg/ calf and in weeks 15-16 ceased completely. Hay was fed until week 14 p.n. In each group, seven animals received a rumen fistula in week 3 p.n. for the purpose of another study not described herein. Animals were slaughtered on day 240 ± 7 p.n. Blood and tissue samples from thymus, spleen, mesenterial lymph nodes and jejunal Peyer´s patches (PP) were dissected and comprised leukocytes were isolated. Further, leukocytes were isolated from the epithelium and lamina propria of dissected mid jejunal and terminal ileal tissue samples. The CD2- (T cells), CD4- (T helper cells), CD8- (T cytotoxic cells) and CD21- (B cells) T and B cell subsets were quantified by flow cytometry. For statistical analysis MIXED procedure of the SAS Enterprise Guide 6.1 was used. The statistical model included the fixed effects feeding group and fistula.

Results: The BW on slaughtering day was higher in heifers fed 20% MR during the pre-weaning period compared to animals fed 10% MR ($p < 0.005$). CD21 B cell subsets were increased ($p < 0.05$) and CD8 T cell subsets tended to decrease ($p < 0.1$) in spleen of non-fistulated heifers fed 20% MR pre-weaning compared to heifers fed 10% MR or those with a fistula. The portion of CD8-positive cells were increased in mesenterial lymph nodes of non-fistulated heifers fed 20% MR compared to heifers fed 10% of BW MR or heifers with a fistula ($p < 0.05$). CD21 B cell subsets in the ileal epithelium tended to be higher in heifers fed 20% MR compared to heifers fed 10% MR ($p < 0.1$). Additionally, fistula significantly influenced the CD4 T cell subsets in the jejunal epithelium ($p = 0.015$) and tended to affect CD4 and CD8 T cell subsets in thymus and CD21 B cell subsets in the ileal lamina propria ($p < 0.1$). There were no effects of fixed factors on T and B cell subsets in blood or PP.

Conclusions: The current study clearly shows long lasting effects of the pre-weaning milk feeding regime on the local adaptive immune system indicated by shifts in T and B cell subsets. Further, the study gives evidence that rumen fistulation modulates the immune status of heifers compared to non-fistulated heifers. The causes of immune modulatory effects by the feeding regimen and the fistula as well as the biological role of these changes have to be investigated in further studies.

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* *Leibniz Institut für Nutztierbiologie, Wilhelm Stahl Allee 2, Dummerstorf, Germany, liermann@fbn-dummerstorf.de*

61.

Effect of hydrolysable tannin treatment and wilting of lucerne on silage composition, short-term dry matter intake and feed preference by goats

Einfluss von hydrolisierbaren Tanninen und Anwelken auf die chemische Zusammensetzung von Luzernesilagen, Kurzzeit-Trockenmasseaufnahme und Futterwahlverhalten von Ziegen

*Hilgers B., Gerlach K., Südekum K.-H. – Bonn

Forage legumes, like lucerne, are significant protein sources for ruminants on farms. However, proteins of ensiled lucerne are often poorly utilized, because of extensive protein degradation during wilting and storage. It has been reported that addition of hydrolysable tannins (HT) can decrease proteolytic processes occurring during ensiling, due to reaction with proteins and inhibition of gram-negative bacteria (1). Nevertheless, it is still unknown how HT, applied to silages in different concentrations of dry matter (DM), affect short-time feed intake and preference of goats.

Methods: A 2 hectare pure stand of lucerne (*Medicago sativa L.*, second cut) was cultivated and cut on 7 May 2018 in the morning at the middle bud stage. To produce silages with two different DM concentrations, one-half was wilted for five hours and the second half for 26 hours over night. Lucerne was ensiled in 120-L barrels with 24% DM for low dry matter silages (L24) and 37% DM for high dry matter silages (L37), respectively. Before ensiling, six silage treatments were prepared, each in quadruplicate. A commercially available HT extract (*Castanea sativa L.)* was added to the L24 and L37 to reach HT concentrations of 0% DM (T0), 2% DM (T2) and 4% DM (T4). At day 115 after ensiling, three barrels per treatment were opened and samples were taken for chemical analyses. Silages were analysed for DM, ash, crude lipids, crude protein (CP), CP fractions, fibre fractions, utilisable CP at the duodenum, fermentation acids, water-soluble carbohydrates, ammonia-N (NH_3-N), pH and alcohols. A preference trial (PT) was conducted with six Saanen-type wethers (mean body weight 91.3 kg (± 2.4 kg)). During an adaptation period (7 days) single meals of each silage and lucerne hay as control were offered in a randomized order. During the subsequent experimental phase (21 days), each possible 2-way combination of the six silages and the lucerne hay was presented side by side in a randomized order. The weight of the forages was determined every 30 min over a total time of 3 h to calculate the initial forage preference and total dry matter intake (DMI). Data were analysed using the GLM procedure of SAS 9.4 with animal and feed as fixed effects and DMI as random effect for the PT and the means were separated using the minimum significant difference from the Waller-Duncan k-ratio t-test.

Results: Fresh lucerne was high in CP (233 g/kg DM) with a share of non-protein nitrogen (NPN) of 268 g/kg CP, which increased during the 26 hours of wilting to 387 g/kg CP. After ensiling NPN concentration was highest in L24T0 (824 g NPN/kg CP). Increase in DM and addition of HT decreased the NPN concentration. The maximum effect was achieved by a combination of both treatments, which caused a decline in NPN of 22% for L37T4 compared to L24T0**.** Results of the PT showed an impact of animal ($P<0.001$) and feed ($P<0.001$) on the 3 h DMI. The highest DMI was determined for L37T0 with 683 g DM/3 h. The addition of HT reduced the DMI of L37T2 and L37T4 to 537 g and 533 g, respectively. The 24T0 showed the lowest DMI with 210 g/3 h, resulting in a decline in DMI of 69% compared to L37T0. Increasing the HT concentration in the L24 to 4% DM enhanced DMI by 76 g DM.

Conclusions: Increasing DM and application of a HT extract improved protein quality of lucerne silages, both separately and in combination. DM had a higher effect on the DMI during the PT than the addition of HT to the lucerne. Tannins only had a positive effect on DMI in the L24.

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* *University of Bonn Institute of Animal Science, Endenicher Allee 15, 53115 Bonn, Germany, bhil@itw.uni-bonn.de*

62.

Growth performance and feed efficiency in broilers following partial replacement of soy bean meal by hatching egg meal

Zur Leistung von Broilern (Zunahmen, Futteraufwand) nach anteiligem Ersatz von Sojaextraktionsschrot durch Bruteipulver

*Bochnia M., Schroeder A., Reitemeier S., Gottschalk J., Einspanier A., Euring B., Moerl L., Idakiev V., Zeyner A. – Halle (Saale) / Leipzig / Magdeburg

More than 47 Mio. male day-old chickens were killed in German hatcheries every year (1). Several methods have been developed for sexing chicken eggs such es endocrinological sex sorting to overcome this situation. Sexed hatching eggs are allowed to be used as feedstuff (here: freeze dried meal from egg contents after 9d hatching; EM) in animal nutrition (EC 1069/2009), whereat the high content of protein and especially sulphur-containing amino acids (2) are advisable for chicken diets (no intraspecies feed ban; art. 14/31 EC 1069/2009) or pigs. Aim of the present study was to investigate the potential of a partial replacement of soybean meal with EM regarding growth performance in chickens.

Methods: In total 240 broiler chickens (Ross 308) were allocated in pens (n = 5/pen) and fed *ad libitum* during the trial. The pens (n = 48) were randomly assigned to the 6 treatments with each 8 pens (T1 – T6) offering a basal pelleted diet to the chickens (wheat, soybean meal, maize, sunflower seed meal extract, rape seed meal, wheat bran, minerals) including in T1 2% soybean meal (SBM), T2 4% SBM, T3 6% SBM, T4 2% egg meal (EM with (g/kg dm) crude protein (CP) 532, crude ash (CA) 38, crude lipids (CL) 360; T5 4% EM, T6 6% EM, with similar nutrient contents in the test diets (dm [g/kg] 900, CP 230, CA 63-66, CL 36-52, crude fiber 54-56, metabolizable energy T1 – T3, 11.9 – 12.2; T4 – T6, 12.4 – 12.7 MJ/kg dm. The diet composition was according to nutrient requirements for broiler chickens (3), but mainly focused on equalized CP and essential amino acid contents. Feed intake and body weight (bwt) of chickens were recorded weekly and feed conversion ratio (FCR) was determined. A mixed model (Proc. GLIMMIX) was used for statistics (SAS 9.4).

Results: Feed intake in broiler chickens was not affected by the different diet compositions. The bw at the beginning of the trial (~ 48 g) was equally distributed over all 6 treatment groups ($P > 0.05$). Chickens fed with T6 (1.588 ± 30.67 g) had a higher final bw than with T3 (1.494 ± 28.47 g, $P < 0.05$) which was also true for the comparison of T1 (1.357 ± 36.25 g) and T4 (1.229 ± 37.07 g, $P < 0.05$). With T5 (1.432 ± 35.65 g) and T6 chickens had a higher final bw than with T4 ($P < 0.05$), whereas within the SBM groups, T3 induced a significantly higher final bw than T1 ($P < 0.05$). The FCR was [kg feed/kg weight gain] decreased reciprocally proportional with level of SBM or EM supplementation: for T1 (1.96 ± 0.09) SBM was a little inferior to T4 (1.92 ± 0.05, $P > 0.05$) similar to T2 (1.89 ± 0.07) and T5 (1.80 ± 0.05, $P > 0.05$) in comparison, whereas T6 (1.68 ± 0.02) showed numerically the lowest FCR and highest final bw (~ 95g more per chicken).

Conclusions: The present study showed that partial replacement of SBM in diets for broiler chickens with EM can achieve similar results regarding growth performance during the fattening stage, whereas with 6% EM the feed conversion rate and the bw gain were particularly higher. However, this might be related to the particularly greater energy intake due to the high fat content of EM. Future studies may test if increasing amounts of EM in diets for broiler chickens can maintain the positive effects, whereas the high fat content may be limitative.

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* *Martin Luther University Halle Wittenberg, Halle (Saale), Germany, mandy.bochnia@landw.uni-halle.de*

63.

Effects of riboflavin concentration in premixes on performance, egg quality and health indicators in laying hens

Einfluss der Riboflavinkonzentration im Prämix auf Leistung, Eiqualität und Gesundheitsindikatoren bei Legehennen

*Leiber F., Holinger M., Maeschli A., Früh B., Lambertz C., Maurer V., Ayrle H. – Frick / Frankfurt

In organic animal feeds, the use of genetically modified organisms is prohibited. Therefore, B-vitamins have to be produced with alternative, more expensive strains of microorganisms and the dosage of added riboflavin is economically relevant (1). Since riboflavin requirement data for poultry are comparably old, the requirements for laying hens with a typical organic diet were reassessed. The aim was to find safe lower thresholds of riboflavin supplementation without impact on animal health and performance.

Methods: Basis and control was a usual Swiss organic layer feed with 4.5 mg riboflavin/kg feed added via the premix (R4.5). Identical feed was produced with 3.0 and 1.5 mg riboflavin/kg feed added (R3.0 and R1.5, respectively). Diets were applied to respectively three groups (pens) of 15 Lohmann Brown-classic hens at 25 weeks of age, when the experiment started. Ad libitum feeding of the diets lasted for 18 weeks. Feed consumption and laying performance were recorded daily, by pen. Individual body weight was determined and assessments for motility behavior, and scores for lesions, plumage, leg integrity and keel bone integrity were carried out seven times throughout the experiment. With the same frequency, eggs (20 per pen) were collected and analysed for yolk riboflavin content, colour, shell strength and weights of yolk, albumen and shell. In experimental weeks 11 and 18, respectively two hens from each pen were slaughtered and livers were analysed for riboflavin concentration. Statistical analyses were performed with R (version 3.5.3; R Core Team 2019). Fixed effects were week and treatment, including their interaction. Random effect was pen. For body weight and scorings, nested random effects were animal within pen. Riboflavin analyses had been carried out on composite samples (two per pen) and respective data were therefore only compared by Students t-test.

Results: Effective total riboflavin concentration at the start of the of the experiment was 6.1, 5.3, and 3.3 mg/kg feed for R4.5, R3.0, and R1.5, respectively. Concentrations in all diets declined during the first eight weeks of the experiment, and subsequently stayed stable at 5.0, 4.5, and 3.0 mg/kg for R4.5, R3.0, and R1.5, respectively. Neither laying performance, nor feed intake, body weight or any health and welfare indicator differed between the three feeding treatments. Egg quality parameters (weight, egg shell stability and yolk colour) were not affected by the level of riboflavin supplementation. However, riboflavin concentrations in egg yolks of the R1.5-treatment declined in experimental week 7 by approximately 20% compared to the other two treatments. Subsequently, the values remained stable until the end of the experiment in week 18 (0.58, 0.56 and 0.43 mg/100g fresh matter for R4.5, R3.0, and R1.5, respectively). Also in the livers of R1.5 animals, we found lower riboflavin concentrations in experimental weeks 11 and 18 (8-10% decline) compared to R4.5 and R3.0 among which no difference occurred (week 18: 2.18, 2.20 and 1.98 mg/100g fresh matter for R4.5, R3.0, and R1.5, respectively).

Conclusions: Supplementation of only 1.5 mg riboflavin/kg feed led to reduced levels in egg yolk and liver, indicating a metabolic deficiency of this water soluble vitamin. Although no impacts on feed intake, body weight, performance and clinical scores were found, this dosage should be considered as too low. The addition of either 3.0 or 4.5 mg did not result in any differences, suggesting that both dosages are above the lower critical threshold. Regarding the effective total riboflavin concentration of diets R3.0 and R1.5, this threshold was between 4.5 and 3.0 mg/kg, which is well in line with former literature (2). The addition of 3.0 mg riboflavin/kg feed is suggested as safe in organic layer nutrition.

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* *FiBL Department of Livestock Sciences, Ackerstrasse 113, 5070 Frick, Switzerland, florian.leiber@fibl.org*

64.

Coccidiostatic effects of two feed additives (coccidiostat vs. oregano oil) in fattening rabbits – results of a field study

Kokzidiostatische Effekte zweier Futtermittelzusatzstoffe (Kokzidiostatikum vs. Oreganoöl) bei Mastkaninchen - Ergebnisse einer Feldstudie

*Lohkamp F., Hankel J., Beineke A., Strube C., Kamphues J. – Hanover

For decades coccidiostats have been used as feed additives in compound feeds for rabbits to combat coccidiosis. According to current EU feed legislation, for rabbits only two coccidiostats are available. Consequently, it is hardly possible to implement long-term substance rotation programmes to avoid development of antiparasitic resistance (as in poultry). The essential oil of oregano (*Origanum vulgare*) has been shown by in vivo studies to have anticoccidial effects against certain species of *Eimeria* in poultry (1; 2; 3). The aim of this field study was to compare oregano oil with a chemical coccidiostat in naturally coccidia-infected fattening rabbits.

Methods: In a fattening rabbit farm, one compound feed with different additives was used. The first group of rabbits (control) did not receive any of the following feed additives, the second group had an oregano oil extract (75 mg / kg) and the third group a coccidiostat (Diclazuril, 1 mg / kg) in the diet. In three rounds, 150 weaned, five week old fattening hybrids each were assigned to three groups of 50 animals kept in a group housing system with fully slatted floors and elevated platforms. Each fattening period lasted six weeks. Performance was monitored by weighing the rabbits at the beginning, in the middle and at the end of this period. Feed consumption was measured on group basis only. Deceased rabbits were necropsied. Every week faeces samples were tested to determine the load by and to identify the species of *Eimeria*. The number of oocysts per gram of faeces (OPG) was counted by the McMaster method. Statistical analysis was performed as two factorial ANOVA of SAS (version 7.1) with "diet" and "week" as fixed factors for OPG values as well as for performance data.

Results: The rate of losses was highest in the oregano oil groups (16.0 %), followed by diclazuril groups (12.7 %) and the control rabbits (12.0 %). The expected increase in fecal oocyst excretion in the first two to four weeks after weaning with subsequent decrease in excretion at the onset of immunity was also observed in the present field study. Diclazuril and oregano oil could attenuate this increase compared to the control group in all three and two rounds respectively. But in all groups the course of fecal excretion and the OPG values varied markedly without significant differences for dietary treatment regarding performance [ADG (g/d): 35.8 - 37.4; FCR (kg/kg): 3.60 - 3.73]. These variations show that, in addition to the feed additives, other factors (stressors) might have influenced coccidia excretion.It has to be underlined that only low to moderate pathogenic *Eimeria* species were identified (*E. magna, E. media, E. perforans, E. exigua*). Thus, it is not astonishing that there was no sign for coccidiosis as the cause of losses. In spite of a higher loss rate in all groups, not in a single necropsied rabbit intensive alterations indicating marked coccidiosis were observed. These findings from the necropsy correlate quite well with the identified species that are considered as low to moderate pathogenic species. Regarding these species the dietary treatment did not result in any 'preventive effect'. Not even the classical coccidiostat proved to be a constantly effective additive in terms of OPG values.

Conclusions: This study confirms that oocyst excretion correlates neither with the loss rate nor with signs of coccidiosis, especially when only low and moderate pathogenic *Eimeria* are present in the stock / in the animal. Coccidia are not in every case the main reason of increased losses. In this context, the *ad libitum* feeding of concentrates, which often leads to dysbioses due to overstrained digestive capacities, should be viewed critically. Their involvement in increased losses is generally underestimated, especially when high oocyst numbers in faeces are detected. It has to be emphasized that the identification of the present *Eimeria* species is obligatory both before conducting efficacy studies and before a therapeutic intervention in a rabbit stock.

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* *University of Veterinary Medicine Hannover Institute for Animal Nutrition, Bischofsholer Damm 15, 30173 Hanover, Germany, florian.lohkamp@tiho-hannover.de*

65.

Impact of water temperature on diet preference for protein in Nile tilapia and effects on whole body composition

Einfluss der Wassertemperatur auf die Protein-Präferenz in Nil-Tilapien und Auswirkungen auf die Gesamtkörperzusammensetzung

*Abd El-Wahab A., Visscher C., Teitge F., Steinhagen D. – Mansoura / Hanover

Currently, tilapia is the second most important cultured fish worldwide, whereas Nile tilapia is the most widely cultured species allover world. Fish, as many different animals, show "nutritional wisdom," having the ability to select from different feed ingredients to form a balanced diet [1]. This study aimed to test preference of diets for a high (38%) or a low protein (30%) content "a choice criterion" tested with two colors depending on water temperatures (22 or 30 °C) "the only variable factor" on whole body composition in Nile tilapia.

Methods: Tilapia fish were allowed to acclimatize to laboratory conditions for at least two weeks. Therafter, at beginning of the experiment theNile tilapia (BW ~38 g) were reared in twelve 200-L tanks for 63 days (12 fish/tank). Two different diets were formulated to contain 30% (low) or 38% (high) crude protein on dry matter (DM) basis. Each level of dietary protein was prepared to have either red (code A) or yellow (code B) color according to [2]. For Nile tilapia, the recommended protein content in the feed for the weight range of 20-200 g is 34% as-fed [3], that is, about 38% based on DM. Because of the weight at the start of the experiment and the expected increase, we selected the two diets (30 and 38% protein). Each aquaria was divided into two parts that allow offering the diets with freely movement of fish. The temperature was adjusted to be either low (22 °C) or high (30 °C) by using thermostatically controlled heaters. Fish were fed for 2 h twice daily at 8:00 and 15:00 h and the uneaten feed were collected. Therafter, feed intake was recorded on daily basis. Individual body weights were measured weekly. At the end of the experiment, a random sample of 48 fish (4 from each tank) was collected to determine the final whole body composition. Body composition of fish was analysed in one pooled sample per tank. Diets were analyzed by standard procedures [4]. The amino acids were measured by ion-exchange chromatography (Biotronic, Germany). Levels of medium- and long-chain fatty acids were determined in tissue samples by carrying out gas chromatography (ThermoScientific®, Germany). The statistical analysis of the data was performed using SAS®9.4. Data were checked according to normal distribution. Differences between groups were analysed by means of the T-test for independent samples ($p < 0.05$).

Results: The percentage of the 38% protein diet intake varied from 26 to 44%. High water temperature resulted in significantly ($p < 0.05$) higher body weight gain (39.2 g ± 4.29) compared to low water temperature (4.75 g ± 0.655). Whole body composition showed no significant differences between DM, protein, and ash contents regarding the water temperature (259 g/kg, 611 and 208 g/kg DM, respectively for low water temperature vs. 265 g/kg, 608 and 184 g/kg DM, respectively for high water temperature). The amino acids profile in whole body composition showed no significant differences between treatments of different water temperatures. Interestingly, about seven different fatty acids of 19 fatty acids of the fish body showed significant differences between low and high water temperatures. Only the palmitic acid level was significantly ($p < 0.05$) higher for fish reared in a high water temperature, namely, 20.5 g/kg DM, than those in a low water temperature (17.4 g/kg DM). Contents of only six specific fatty acids (Arachidic acid, Gadoleic, Dihomo-γ-linolenic acid, Eicosatrienoic acid, Eicosapentaenoic acid, Docosahexaenoic acid) in fish body were significantly higher for low water temperature than those with high water temperature (0.38, 2.45, 1.0, 0.31, 0.85, 5.58% vs. 0.34, 1.77, 0.57, 0.26, 0.64, 3.86% for low and high water temperatures, respectively).

Conclusions: At low water temperatures, even less protein was ingested. The fatty acids composition but not amino acids of fish well reflected the effect of water temperature.

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* *Mansoura Univertsity Faculty of Veterinary Medicine, Nutrition and Nutritional Deficiency Diseases, 35516 Mansoura, Egypt, amrabdelwahab37@yahoo.de*

66.

Influence of diet and strain on urine pH in laboratory mice

Einfluss von Futter und Genetik auf den Harn pH von Labormäusen

*Böswald L. F., Matzek D., Popper B., Kienzle E. – Oberschleißheim / Martinsried

The dietary cation anion balance (DCAB) is known to influence the urine pH of several species (e.g. cats, dogs, cattle, swine) (1). Dietary interventions aiming at the DCAB can influence the metabolic/physiologic state of the animal. The aim of the present study was to test the influence of the DCAB on the urine pH of laboratory mice fed different maintenance diets.

Methods: Two commercially available pelleted diets for laboratory mice in maintenance were analysed for major minerals (calcium [Ca], phosphorus [P], magnesium [Mg], potassium [K], sodium [Na], chloride [Cl], sulphur [S]). The DCAB was calculated as follows: DCAB (mmol/kg dry matter [DM]) = 49.9*Ca + 82.3*Mg + 43.5*Na + 25.6*K – 59*P – 62.4*S – 28.2*Cl with the mineral contents in the unit g/kg DM. Mice (male, 8 weeks old) were fed either diet A (DCAB = 317mmol/kg DM) or diet B (DCAB = 406 mmol/kg DM). Two strains of mice were used (C57Bl6/J; CD1), resulting in four experimental groups: C57Bl6/J-A (n=12), C57Bl/6-B (n=12), CD1-A (n=12), CD1-B (n=12). They were housed in Typ II long IVCs and had ad libitum access to food and water. After two weeks of adaptation to the respective diets, pH was measured from spontaneously secreted urine once per week during an eight-week period of testing. Influence of diet and strain on urine pH was tested (two-way ANOVA using SigmaPlot software; significance level: $p<0.05$).

Results: In the mice from strain C57Bl6/J fed diet A (lower DCAB), urine pH was 5.57±0.18 (mean±SD), while it averaged 6.16±0.19 with diet B (higher DCAB). The feed effect was similar in the CD1 mice, resulting in mean pH values of 6.08±0.12 with diet A and 6.87±0.47 with diet B. There was a significant influence of diet and of strain on urine pH ($p<0.01$), while these factors did not interact ($p=0.37$). The C57Bl6/J mice (inbred strain) showed lower urine pH values than the CD1 mice (outbred stock). This might be due to yet unknown genetic differences in the physiology related to mineral transport in the kidneys.

Conclusions: The results of the present study show that urine pH is influenced by DCAB in mice like in many other species. Because these strains are commonly used for a wide variety of experimental models, potential differences should be investigated further so that they can be taken into account in the planning of animal experiments and interpreting their results, especially when focused on the urinary tract and mineral metabolism. It has to be noted that no information on DCAB was given for the commercial standard diets and thus differences were not obvious for researchers who are not primarily animal nutritionists. Yet, the DCAB led to considerable and biologically relevant differences in urine pH in this study. Commercial diets for laboratory animals should be labelled with information on the DCAB.

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* *Ludwig-Maximilians-Universität München Chair of Animal Nutrition and Dietetics, Oberschleißheim, Germany, linda.boeswald@lmu.de*

67.

Impact of a maternal supplementation of 10% inulin/FOS to a high-protein or high-fat diet during pregnancy and lactation in mice on body weight gain, glucose tolerance, mammary gland development and offspring body weight

Einfluss einer Inulin/Oligofructose Supplementierung zu einer Hochprotein- oder Hochfettdiät während der Trächtigkeit und Laktation in Mäusen auf Körpergewichtszunahme, Glukosetoleranz, Milchdrüsenentwicklung und Körpergewicht der Nachkommen

*Koch F., Langhammer M., Tuchscherer A., Hammon H. M., Mielenz M., Kuhla B., Metges C. C. – Dummerstorf

An imbalanced maternal diet with high fat (HF) or high protein (HP) concentrations consumed during pregnancy and lactation may induce morphological and functional changes of the mammary gland (MG) and impair offspring development (1). Prebiotic supplements, e.g. oligofructose (FOS) and inulin beneficially affected gut microbiota and reduced body fat mass (2). Prebiotics may be beneficial for MG function and thus may influence the development of the offspring (3). The aim of the present study was to elucidate the impact of 10% inulin/FOS supplementation to a maternal HF or HP diet in mice on maternal body weight (BW) gain, glucose tolerance, morphological and physiological development of the MG and offspring´s BW.

Methods: Five-week-old female C57BL/6NCrl mice were housed individually at 22°C with a 12-h light-dark cycle and *ad libitum* feeding. Mice received a control diet (CD; AIN-93G; 16.2 MJ gross energy per kg) for 2 weeks. Thereafter, 64 mice were randomly allocated to 1 of 6 experimental diets: CD, HF (45% energy from fat; 19.8 MJ/kg) and HP (40% protein; 16.0 MJ/kg) without and with 10% inulin/FOS (1:1) supplementation (CD+I, HF+I, HP+I) during pregnancy and lactation. Body weight and food intake were recorded every second day (d) starting 7 d before mating. Female mice were mated at 8 weeks of age. On d 15 of pregnancy, an intraperitoneal glucose tolerance test was performed (1 g glucose/kg BW). After birth, litters were standardized to 5 pups and their BW was recorded until d 9. Dams were killed on d 10 of lactation to perform whole mount staining and determine MG morphology. Mammary gland subsamples were utilized for mRNA abundance of *Wap*, *Csn2* (synthesis of milk proteins), *Dgat1*, *Cidea*, *Xdh* (synthesis of milk lipids), *Lalba*, *Gale* and *B4galt1* (lactose synthesis) relative to the reference genes *Actb* and *Ywhaz*. Data analysis was performed by repeated measure ANOVA using the MIXED procedure of SAS (Version 9.4) including the fixed factors diet, time, the interaction between diet and time, and multiple comparisons using the Tukey test.

Results: Food intake did not differ, but energy intake was higher in HF than CD, CD+I, HP and HP+I ($P<0.01$) from 2 d before mating onwards. Energy intake tended to be higher in HF+I than CD, CD+I and HP+I ($P=0.07$), but did not differ between HF and HF+I. Maternal BW gain during pregnancy was higher in HF and HF+I than CD ($P<0.001$), CD+I ($P<0.05$) and HP ($P<0.001$). HP+I gained more BW during pregnancy than HP ($P<0.05$). Glucose tolerance in HP and HP+I was disturbed compared to CD and CD+I ($P<0.05$). On d 10 of lactation, BW and MG morphology did not differ among the dietary groups. Mammary gland *Dgat1* mRNA abundance tended to be 23-25% lower in HF and HF+I ($P<0.07$) than CD. *Lalba* mRNA abundance was 1.7-fold higher in HP+I than HP ($P<0.05$) and tended to be 1.6-fold higher in HP+I than CD+I ($P=0.09$). *B4galt1* mRNA abundance tended to be 16-22% lower in CD+I ($P=0.08$) and HF ($P=0.09$) than CD, whereas in HP and HP+I, *B4galt1* mRNA abundance was 23-25% lower than in CD (each $P<0.05$). All other mRNA abundances showed no diet effect. Body weight gain of 9-d old pups was greater when dams were fed HF and HF+I diets relative to all other diets (each $P<0.05$).

Conclusions: Supplementation of inulin/FOS to a HP diet induced BW gain during pregnancy, but did not improve glucose tolerance when compared with HP. Both HP and HP+I indicated to regulate lactose synthesis without altering MG morphology. Higher energy content in HF and HF+I diets induced maternal and offspring BW gain and lower milk lipid and lactose synthesis. Irrespective of the basal diet, inulin/FOS did not affect offspring growth.

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* *Leibniz Institute for Farm animal biology Institute of Nutritional Physiology "Oskar Kellner", Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany, koch@fbn-dummerstorf.de*

68.

High-concentrate diet differently affects plasma metabolomic profile of primiparous and multiparous dairy cows in early lactation

Erhöhte Kraftfuttergabe beeinflusst das Stoffwechselprofil von primiparen und multiparen Kühen in der Frühlaktation unterschiedlich

*Reisinger N., Stauder A., Schwartz-Zimmermann H., Neubauer V., Humer E., Petri R. M., Zebeli Q. – Tulln / Vienna / Krems

Primiparous (PP) cows are more likely to experience negative consequences on health status during early lactation than multiparous (MP) cows, especially when fed diets rich in concentrates. We hypothesized that high concentrate diets during early lactation might be a greater challenge for PP cows, likely also leading to different regulations of key metabolic pathways. Therefore, the aim of the study was to compare the metabolomic profile of PP and MP cows in response to a challenge diet.

Methods: For the study twenty-four Simmental cows were used. The group consisted of eight primiparous cows and sixteen multiparous (51 ± 24 DMI). Cows were first fed a baseline diet for two weeks (60% roughage; 40% concentrate). Thereafter, cows were fed a challenge diet for four weeks (; 40% roughage; 60% concentrate). Blood samples from all animals were collected during the baseline diet as well as during week 1 and 4 of the challenge diet. Plasma was stored at -80°C until analysis. Biocrates MxP® Quant 500 kit was used for analysis of approximately 500 plasma metabolites. A 6500+ QTrap from Sciex was used for tandem mass spectrometric analysis. Statistical analysis was performed by using the MIXED procedure of SAS, including phase, parity and their interaction as fixed effects. Multivariate analysis was performed using Meta-Analyst software.

Results: Principal coordinate analysis of all metabolites showed that PP cows clustered separately from MP cows. VIP score revealed that the following metabolites had the greatest effect on separation of the PP and MP cows: acylcarnitines (acetylcarnithine, propionylcarnitin), glutamate, amino acid related metabolites (betaine, proline betaine, cis-4-hydroxyproline, trans-4-hydroxyproline), lysophosphatidlycholines (lysoPC a C16:0, lysoPC a C16:1) as well as phosphatidlycholines (PC aa C28:1, PC aa C36:0, PC aa C42:6, PC aa C34:2). Glutamate, cis-4-hydroxyproline and trans-4-hydroxyproline were higher in PP cows ($P < 0.001$), whereas betaine, proline betaine and myristic acid were lower in PP cows ($P < 0.05$) compared to MP cows. Glutamate concentration was decreasing during challenge week 4 compared to challenge week 1 in both PP and MP cows ($P < 0.05$). Cis-4-hydroxyproline was decreased in challenge week 1 and 4 in PP cows ($P < 0.05$) and in challenge week 4 in MP cows ($P < 0.05$). Trans-4-hydroxyproline was decreased in challenge week 1 and week 4 in PP and MP cows ($P < 0.01$). Both actylcarnithines were higher in PP cows than in MP cows ($P < 0.001$). Acetylcarnithine was lower during challenge week 1 ($P < 0.05$) whereas propionylcarnitin was significantly higher during challenge week 1 ($P < 0.05$). Both lysophosphatidlycholines were lower in PP cows compared to MP cows ($P < 0.01$). LysoPC a C16:0 was higher in MP cows in challenge week 4 compared to week 1 ($P < 0.001$). PC aa C28:1 and PC ae C34:2 were lower in PP cows compared to MP cows ($P < 0.05$), whereas PC aa C42:6 was higher in PP cows ($P < 0.01$). PC aa C36:0 was lower in both PP and MP cows in challenge week 1 and 4 compared to the baseline ($P < 0.01$). PC aa C42:6 was decreased in challenge week 1 and 4 compared to the baseline ($P < 0.01$) whereas PC aa C34:2 was increased in challenge week 4 compared to the baseline ($P < 0.001$).

Conclusions: Our data suggest large differences in the responses of immunometabolic variables between PP and MP cows fed a challenge diet. These data confirm results of earlier investigations on liver health and acute phase response. As energy and nutrient balance was similar in both groups of cows, it is reasonable to assume that an altered metabolomic profile in PP cows might be indicative for their higher susceptibility to metabolic and/or immunometabolic disorders during early lactation.

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* *BIOMIN Research Center, Technopark 1, 3430 Tulln, Austria, nicole.reisinger@biomin.net*

69.

Efficacy of a mixture of mono- and diglycerides and probiotic on performance and gut health in broilers from 1 to 36 days of age after a challenge with *Clostridium perfringens*

Wirksamkeit einer Mischung aus Mono- und Diglyceriden und Probiotika auf Leistung und Darmgesundheit bei Broilern im Alter von 1 bis 36 Tagen nach einer Exposition mit Clostridium perfringens

*Martínez-Vallespín B., Männer K., Ader P., Zentek J. – Berlin / Lampertheim

Necrotic enteritis (NE), a disease resulting from infection with *Clostridium perfringens* strains expressing NetB toxin, is responsible for high losses in broiler production globally. High efforts are being done in searching for strategies or products that could moderate its effects. This study investigated the efficacy of a mixture of mono- and diglycerides, which have been proven as effective microbicidal against different pathogens (1), without or with combination of C*lostridium butyricum* (probiotic), supplemented to basal diets fed to broilers exposed to an experimental *Clostridium perfringens* infection.

Methods: 200 one day(d)-old healthy male broiler chickens (Cobb) were randomly distributed in 4 groups and allocated into 24 pens containing 8 - 9 birds per pen (6 replicates per group) until d 12. On d 13, 36 birds per treatment were transferred to cages housing 3 animals until the end of the study at d 36. During the experimental period three basal mash feed diets (based on wheat, soybean meal and maize) were presented: i) d 1 to d 12 (starter diet), ii) d 13 to d 24 (grower diet), iii) d 25 to d 36 (finisher diet). On d 18, 19 and 20, the animals were orally challenged with *Clostridium perfringens* (0.5 ml with $5x10^8$ cfu), except for a negative control group (T1). The other treatment groups included a non-supplemented positive control (T2), the supplementation of 3000 mg/kg of a mixture of mono- and diglycerides of butyric-, capric- and caprylic acid from d 1 to d 36 (T3) and the supplementation of 3000 mg/kg of the mono- and diglycerides mixture from d 1 to d 12 exchanged by $2.5x10^8$ cfu/kg of *Clostridium butyricum* (CBM588) at d 13 and until d 36 (T4). To induce mild immunosuppression, birds were vaccinated orally on d 4 and d 9 of age with an attenuated vaccine for Infectious Bursal Disease (Poulvac Bursa Plus®, Zoetis, Belgium), and, on d 14 and d 16, with a tenfold dose of 2 vaccines containing viable, sporulated *Eimeria* oocysts of different lines of *coccidia* (Hipracox®, Hipra, Belgium and Paracox-8®, MSD Animal Health, Belgium). Body weight (BW) and feed intake (FI) were recorded at the beginning of the experiment and at d 7, 12, 19, 24, 31 and 36. General health status was daily checked. For examination of coccidial and NE lesions, ten birds were randomly taken at d 17 (remaining floor birds), and at d 22 (cage birds). All remaining birds per cage were finally slaughtered at d 36. Animals were killed after stunning by cervical dislocation. Duodenum, jejunum, ileum, and caeca were examined for scoring of coccidial and NE lesions. The statistical analyses were done with SPSS (IBM SPSS Version 21) and based on one-way ANOVA ($p<0.05$).

Results: Overall mortality rate was on average 26.3%, being the highest in T2 (50%). Mortality was reduced by the supplementation of the mono-and diglycerides mixture with or without the probiotic (30% and 25%, for T3 and T4, respectively). No mortality was observed in T1. Performance parameters of T3 and, especially, T4 showed values similar to T1 during most of the experimental period whereas same parameters were significantly lower in T2 ($p\leq0.05$). At d 17 and 22, all animals showed similar coccidial lesions in duodenum, jejunum, ileum and caecum, mainly scattered petechiae and white spots, which almost disappeared at d 36. Except for jejunum and ileum at day 22, these lesions were in tendency slightly less pronounced in T3 and T4 compared to T2. All challenged groups showed similar score of NE lesions, represented by thickening of mucosa with haemorrhagic foci or diffuse red haemorrhagic areas with oedematous mucosa. At d 36 almost no lesions were observed.

Conclusions: The selected infection model was able to clearly demonstrate that the infection with *Clostridium perfringens* leads to a significant impairment of the performance and health of broilers. The mixture of mono- and diglycerides showed a positive effect in protecting the animals against NE, being amplified by the exchange to the probiotic *Clostridium butyricum* in the growing and finishing period. This improvement in health was also clearly reflected in the performance parameters.

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* *Freie Universität Berlin Institute of Animal Nutrition, Königin-Luise-Str. 49, 14195 Berlin, Germany, beatriz.martinezvallespin@fu-berlin.de*

70.

First quantification of propionic acid in serum of dairy cows from different farms

Bestimmung von Propionsäure im Serum von Rindern unterschiedlicher Betriebe

*Lienhart F., Hoedemaker M., Höltershinken M. – Hanover

Propionic acid is produced by microbial fermentation of carbohydrates in the rumen of cattle (up to 54 mol/day). The liver metabolizes up to 75 % of absorbed propionic acid from the rumen and up to 25 % is directly metabolized by mammary gland tissue. Propionic acid is converted to glucose by two key enzymes (Propionyl-CoA Synthetase, EC 6.2.1.17; Propionyl-CoA Carboxylase, EC 6.4.1.3). The inhibition of gluconeogenic enzymes by phenolic substances in vitro was found in ovine hepatocytes (1). Therefore, it is possible that propionic acid accumulates in bovine blood by continuous high production in the rumen. Both very low and high concentrations of propionic acid in peripheral venous blood can be expected. No specification concerning levels of propionic acid in blood except experimental data are available from previous studies.

The aim of this study was to analyze the variability of propionic acid in serum of dairy cows from different farms by gaschromatography.

Methods: A total of 360 serum samples from dairy cows were examined for the presence of propionic acid. Of these samples, 180 were from chronically sick and 180 from healthy animals of various control- and case-farms. Case-farms are dairy farms with a chronic, unspecific disease process. The method is based on the derivatization of propionic acid by reaction with 2-chloroethyl chloroformate (2). The internal standard n-valeric acid was added to the serum and was deproteinized with highly concentrated hydrochloric acid and subsequently centrifuged. For the following treatment, samples were alkalized with sodium hydrate and vacuum-dried. Afterwards the reaction medium consisting of pyridine, acetonitrile and 2-chloroethanol was added to the dried sample. The 2-chloroethyl ester of propionic acid is formed by addition of 2-chloroethyl chloroformate. In the ultimate step, the derivative was extracted by chloroform and analyzed by gaschromatography. N-valeric acid was developed as internal standard (determined limit of detection for propionic acid: 45.26 µmol/l; CV in series for n-valeric acid: 5 %; CV in series for propionic acid: 4.4 %). In addition, all samples with propionic acid contents under 45.26 µmol/l were analyzed with a modified method that aimed at lowering the detection limit. In these samples, the concentration of propionic acid ranged from 0.0001 to 0.004 µmol/l.

The statistical analysis was performed with SAS Enterprise Guide (Wilcoxon's two-sample Test). To determine the reference limits for propionic acid the internationally acknowledged procedure of the IFCC (3) was used. The reference interval should contain the central 0.95 fraction (or 95 %) of the reference distribution.

Results: There were no significant differences in propionic acid concentrations between diseased and healthy cattle within one case and control farm ($P>0.05$). There were significant differences in propionic acid concentrations between diseased cattle within different case and control farms as well as between healthy cattle within different case and control farms ($P<0.05$).

The 2.5th and 97.5th percentile of the whole sample collective incorporates propionic acid concentrations from 0.01 to 1063 µmol/l.

Conclusions: The established method is cost-efficient (no ultra-filtration) and allows easy preparation and rapid processing. Under the given conditions, a first potential normal range for propionic acid in serum of cattle was elaborated: 0.01-1063 µmol/l.

The high variability of propionic acid contents in the investigated collective can be explained by the factors that affect propionic acid levels in blood: the composition of the diet, the dry matter intake and the time of sampling after feed intake. Further studies are necessary to investigate the importance of those factors in order to define proper sampling schemes and to further examine the role of propionic acid as potential biomarker for animal health.

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* *Stiftung Tierärztliche Hochschule Hannover Klinik für Rinder, Bischofsholer Damm 15, 30173 Hanover, Germany, fabienne.lienhart@tiho-hannover.de*

71.

Effect of 3-Nitrooxypropanol and varying concentrate feed proportions in the ration on methane emissions, ketone bodies in blood serum and energy balance in peripartal dairy cows

Einfluss von 3-Nitrooxypropanol und variierendem Kraftfutteranteil in der Ration auf Methanemission, Ketonkörper im Blutserum und Energiebilanz bei Milchkühen im peripartalen Zeitraum

*Steinmetz M., von Soosten D., Hüther L., Frahm J., Kersten S., Meyer U., Zeyner A., Dänicke S. – Braunschweig

Methane from enteric fermentation in ruminant livestock contributes approximately 17% of total anthropogenic methane emissions. Methanogenesis is a major ruminal H_2 sink, but causes a loss of 2 – 12% of gross energy intake (Johnson and Johnson, 1995). Transition cows are challenged with enhanced lipolysis when energy demand exceeds energy supply. 3-Nitrooxyproanol (**NOP**) inhibits methane formation by targeting the active site of methyl coenzyme-M reductase (Duval and Kindermann, 2012). We hypothesized that higher concentrate feed proportions, NOP supplementation, and their potential synergism in the ration reduce CH_4 emissions, ketone bodies and non-esterified fatty acids in blood while energy balance is enhanced due to decreased energy loss from methanogenesis.

Methods: From d 28 *antepartum* (**a.p.**) until d 120 *postpartum* (**p.p.**), a feeding trial was conducted using 55 pluriparous German Holstein cows which were grouped in a 2x2 factorial design by low (**CL**) or high (**CH**) concentrate (**C**) feed proportion in the ration and NOP (48.4 mg NOP/kg dry matter intake (**DMI**) in NOPCL group and 51.2 mg NOP/kg DMI in NOPCH group) or placebo (**CON**) supplementation. Cows received a partial mixed ration (**PMR**) of 70% maize silage, 20% grass silage, and 10% concentrates on DM basis in weighing troughs *ad libitum*. Additional concentrates were fed by automatic feeders. CL and CH groups received 15% and 40% concentrates in the ration a.p., resp., whereas C proportion increased to 30% for CL groups, and for CH groups gradually from 30 to 55% p.p. NOP was supplied via concentrates from PMR and automatic feeders. Emitted CH_4 was quantified using the GreenFeed system. Milk yield and composition were measured daily and twice weekly, resp. Blood serum samples were prepared from jugular vein blood on d 28, 14, 7, 3 a.p. and d 1, 3, 7, 14, 21, 28, 35, 49, 73, 98, 120 p.p. to analyze ß-hydroxybutyrate (**BHB**) and non-esterified fatty acids (**NEFA**) photometrically. Statistics were performed using PROC MIXED in SAS v9.4 with NOP, C, time, and their interactions as fixed effects, cow as random effect, and time as a repeated measure.

Results: Dry matter intake (**DMI**) was not affected by NOP, but higher for CH groups and influenced by calving. Energy-corrected milk yield (**ECM**) increased p.p. and was significantly lower for NOPCH as compared to CONCH, while NOPCL and CONCL did not differ. CH_4 emissions (g CH_4/day), yield (g CH_4/kg DMI) and emission intensity (g CH_4/kg ECM) were mitigated by NOP (23% for NOPCL, 33% for NOPCH on average), high concentrate feed proportion (11% for CON, 18% for NOP groups on average), and decreased from week 1 a.p. to week 1 p.p. However, under the conditions of the present experiment, the CH_4 emissions of NOPCL group increased to the level of CON groups from week 12 until the end of trial. NEFA were reduced in NOP groups ($P < 0.001$), but unaffected from C proportion and its characteristic peak was observed on d 1 p.p. In contrast, BHB was lower for CH groups from d 14 p.p. until d 73 p.p., but not consistently affected by NOP ($P = 0.096$). Net energy balance (**NEB**) was higher for CH groups over the trial period. Besides, NEB was affected by NOP and concentrate interaction insofar as NEB for NOPCH was higher when compared to CONCH, but not different between NOPCL and CONCL.

Conclusions: We confirmed our hypothesis that supplementing high concentrate proportions and NOP in the diet mitigate methane emissions. NEFA were reduced by NOP, but unaffected from concentrate proportion. BHB was partially decreased in CH groups. Energy deficit was reduced in CH groups and affected by NOP only in combination with high concentrate proportions.

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* *Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health Institute of Animal Nutrition, Bundesallee 37, 38116 Braunschweig, Germany, matthias.steinmetz@fli.de*

72.

Effects of 3-Nitrooxypropanol and varying concentrate feed proportions in the ration on mobilization of fat depot masses in dairy cows

Einfluss von 3-Nitrooxypropanol und variierendem Kraftfutteranteil in der Ration auf die Mobilisierung von Fettdepotmassen bei Milchkühen

*Steinmetz M., von Soosten D., Meyer U., Zeyner A., Dänicke S. – Braunschweig

Excessive body fat lipolysis may lead to liver fat syndrome and ketosis which often occur in transitional dairy cows when energy demand exceeds energy supply due to reduced feed intake. Up to 12% of gross energy intake get lost through enteric methanogenesis. 3-Nitrooxypropanol (**NOP**) inactivates methyl coenzyme-M reductase and inhibits methane formation (Duval and Kindermann, 2012). We hypothesized that varying concentrate feed proportions, NOP supply, and their potential synergism in the ration reduce mobilization of body fat from different adipose tissues due to reduced energy loss.

Methods: From d 28 *antepartum* (**a.p.**) until d 120 *postpartum* (**p.p.**), 55 pluriparous German Holstein cows were allocated in a 2x2 factorial design by low (**CL**) or high (**CH**) concentrate (**C**) feed proportion in the ration and NOP (48.4 mg NOP/kg dry matter intake (**DMI**) in NOPCL group and 51.2 mg NOP/kg DMI in NOPCH group) or placebo (**CON**) supplementation. A partial mixed ration (**PMR**) of 70% maize silage, 20% grass silage, and 10% concentrates on DM basis was fed in weighing troughs *ad libitum*. Via automatic feeders, CL and CH groups received additionally 15% and, resp., 40 % C in the ration a.p., whereas C proportion increased to 30% for CL groups, and gradually from 30 to 55% until d 21 p.p. for CH groups. NOP was supplied via C from PMR and automatic feeders. NOP supplementation reduced CH_4 emissions by about 23% in NOPCL and 33% in NOPCH group (Steinmetz et al., this issue). Body Condition Score (**BCS**) was monitored weekly (Edmonson et al., 1989). On d 3 and 28 p.p., back fat thickness (**BFT**) and rip fat thickness (**RFT**) were measured ultrasonographically while masses of subcutaneous (**SAT**), retroperitoneal (**RAT**), mesenteric (**MAT**), and omental (**OAT**) adipose tissue were determined based on ultrasonographic measurements and associated estimation equations acc. to Raschka et al. (2016). Net energy balance (**NEB**) from d 3 until d 28 p.p. was calculated by subtracting energy requirements for milk production and maintenance from net energy intake. Energy yield per kg mobilized adipose tissue (**EMAT**) was calculated based on the assumption that 1 kg of body fat yields 39.8 MJ gross energy, whereby 16% is lost as heat. The quotient of EMAT and NEB was estimated.Statistics were performed using PROC MIXED in SAS with NOP, C, time, and their interaction as fixed effects, cow as random effect and time as a repeated measure.

Results: Fat was mobilized from all adipose tissues and groups from d 3 until d 28 p.p. ($P < 0.001$). However, CH groups tended to mobilize to a higher extent (on average -18 kg or -34%, resp.) as compared to CL groups (on average -15 kg or -31%, resp.) ($P = 0.084$). Fat depot mass mobilization was unaffected by NOP irrespective of concentrate feed proportion. MAT was mobilized to the highest degree (-36%; $P = 0.003$), followed by SAT and OAT (both -24%), whereas RAT was the least mobilized fat depot (-16%; $P < 0.001$). On average, energy supply from fat mobilization per day was 21.3 MJ NEL for CL, and 23.8 MJ NEL for CH groups, which covered the energy deficit by 44.2% (CONCL), 69.5% (CONCH), 51.3% (NOPCL), and 54.9% (NOPCH). BCS was only affected by time relative to parturition ($P < 0.001$) and decreased from week 1 a.p. until week 4 p.p.

Conclusions: We rejected our hypothesis as mobilization of fat depot mass was unaffected by concentrate feed proportion and NOP. During early postpartal period, energy deficit was only partially compensated by energy mobilization from the investigated adipose tissues. Further supply of energy to cover the energetic deficit may be attributed to fat mobilization from other adipose tissues as well as muscle protein and fat catabolism, but has to be investigated in future experiments.

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* *Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health Institute of Animal Nutrition, Bundesallee 37, 38116 Braunschweig, Germany, matthias.steinmetz@fli.de*

73.

Milk fatty acids are associated with blood metabolites indicating increased body fat mobilization and subclinical ketosis in dairy cows: a meta-analysis

Milchfettsäuren als Indikatoren der Körperfettmobilisierung und der subklinischen Ketose bei Milchkühen: Eine Meta-Analyse

*Khiaosa-ard R., Kleefisch M.-T., Klevenhusen F., Zebeli Q. – Vienna / Berlin

Milk fatty acids (FA) come from several origins including de novo mammary synthesis, dietary origin, rumen origin and mobilization of adipose tissue and each origin predominates the proportions of certain FA in the milk fat. Therefore, milk FA not only represent the nutritive quality of the milk but also reflect the metabolic status of a cow. There have been initiatives (1) for finding milk FA indicators for circulating non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) but the variations among diet regimens as well as cows have not been considered. We took the opportunity to evaluate the potential of using milk FA as indicators for cows' metabolic status using the data from our experiment in transition cows (Study 1) and a meta-analysis (Study 2).

Methods: Raw data of the study in transition cows of our group (2, 3) were used in Study 1. Following the previous report (1), the potential milk FA candidates (4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 16:1 c9, 17:0, 18:1 *c*9, the sum of 4:0 to 12:0 (smSFA), and de novo FA (smSFA + 14:0 + half of 16:0) were evaluated against the concentrations of NEFA and BHB using correlation analysis and logistic regression analysis. The ratios of FA showing the most negative and positive (Pearson) correlations with both serum lipids as well as the milk fat to protein ratio were selected for quantitative analyses (linear and non-linear regressions). Next, the selected milk FA ratios were validated using a meta-analysis approach (Study 2). The publication search was performed on the Web of Science database between year 1900 – 2019. There were 19 qualified studies (81 treatments) included in Study 2. Data analyses of Study 1 and 2 were performed using multiple SAS procedures (PROC CORR, PROC LOGISTIC, PROC MIXED, and PROC NLMIXED). Each mixed model included a continuous predictor (FA ratio) and cow or study was specified as random effect. For the meta-analysis, the number of animals per treatment was used to weight the means reported. The non-linear model was fitted following exponential decay function. The best fit model was chosen based on the Akaike information criterion.

Results: In Study 1, 15:0 showed the highest correlation (-0.65, $P < 0.001$, n = 96) with NEFA and BHB. In good agreement, the logistic regression analysis indicated 15:0 as FA best classifying the events with increased body fat mobilization (NEFA > 0.7 mmol/L) and subclinical ketosis (BHB > 1.2 mmol/L). De novo FA and 14:0 showed slightly lower correlation (-0.52, $P < 0.001$) as did 18:1 *c*9 (0.58, $P < 0.001$). Still, all milk FA showed greater correlations with serum lipids than the fat to protein ratio (max 0.29, $P < 0.05$). For Study 1, the serum lipids showed an exponential decay over an increase of 15:0 to 18:1 *c*9, 14:0 to 18:1 *c*9 and de novo FA to 18:1 *c*9 ratios (R^2= 0.841, 0.833 and 0.846, respectively), and the aforementioned critical NEFA and BHB concentrations were predicted when the ratios fall below 0.02, 0.15 and 0.8, respectively. The fitted models for BHB (n = 69) in the meta-analysis support the findings observed in Study 1 with slight shifts in the rate and horizontal asymptote which was lower in the meta-analysis. Based on the narrow NEFA range in the database (0.1 – 0.8 mmol/L, lowest n = 34), the data was fitted with a linear regression with a negative coefficient ($P < 0.001$) for all FA ratios.

Conclusions: Both Study 1 and 2 suggest that the tested milk FA could be a non-invasive tool to indicate cows with increased risk of metabolic disturbances. Despite different diets and management among the trials, the robust relationships between milk FA ratios and BHB were found.

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* *University of Veterinary Medicine Vienna Institute of Animal Nutrition and Functional Plant Compounds, Vienna, Austria, ratchaneewan.khiaosa-ard@vetmeduni.ac.at*

74.

Effect of a clay mineral-based product on the plasma/blood-lipid profile of early lactating dairy cows fed high-concentrate diets

Einfluss von Tonmineralien auf das Fettstoffwechselprofil im Blut von Kühen in der Frühlaktation nach einer erhöhten Kraftfuttergabe

*Petri R. M., Stauder A., Neubauer V., Schwartz-Zimmermann H., Humer E., Reisinger N., Zebeli Q. – Vienna / Tulln / Krems

To fulfill the high-energy requirements of early lactating cows, often high-concentrate diets are fed in the form of concentrates. However, this can negatively affect the rumen and hindgut ecosystem with consequences for liver health and metabolism. These effects are often more pronounced in primiparous cows due to the first exposure to such high-energy diets, resulting in changes to the incidence of acidosis(1), and consequently higher microbial toxin levels. Clay mineral-based products (CM) have been previously shown to mitigate some of the negative effects of high-concentrate feeding by counteracting toxins within the gastrointestinal tract. However, little is known about the impact of CM on the systemic profile of metabolites based on changes in the gastrointestinal absorption and liver metabolism of microbial toxins. The aim of this study was to investigate if a CM can counteract negative effects of a concentrate-rich diet on the lipid-metabolomic profile of both primiparous and multiparous cows in early lactation.

Methods: Twenty-four lactating Simmental cows (51 ± 24 days in milk, 8 primiparous, 16 multiparous) were fed a baseline diet for two weeks (BASE1-2, 40% concentrate) followed by a high-concentrate diet for 4 weeks (HC1-4, 60% concentrate). Animals were assigned to two different groups either receiving the diet with a CM from BASE2 onwards or with no additive (CON; n = 12 cows per treatment). Blood samples were taken in BASE2, as well as in HC2 and HC4. Samples were analyzed using the Biocrates MxP® Quant 500 kit accounting for approximately a total amount of 500 plasma metabolites including fat, protein, and carbohydrate metabolism. Analysis was performed using a 6500+ QTrap from Sciex. Multivariate analysis was performed using Meta-Analyst software for principle coordinate analysis and variable importance in the projection (VIP) scoring. Statistical analysis was performed using the MIXED procedure of SAS for all metabolites with the additive (CON, CM), parity, and the interaction between them, as fixed effects separated by feeding phase.

Results: Principal coordinate analysis of all metabolites revealed two clusters based on feeding groups CON vs. CM with the first three principle components accounted for 32% of the total variation within the population. Similarly, clustering occurred with relation to feeding phase, with BASE2 clustering farthest away from HC4, and HC1 clustering in between these groups, with the first three principle components accounting for 39% of variation between samples. Multivariate analysis for VIP scores, revealed that various classes of triglycerides (TG) were the most significantly important variables in the separation, and the majority were increased in the cows fed CM compared to the CON diet, whereas phosphatidylcholines (PC) differed most during the BASE2 and HC4 feeding phases, independent of parity. From those TG determined of importance from the VIP scores, the TG 17:0-32:1 was significantly increased in the CM fed cows ($P < 0.02$) during BASE2 feeding. Whereas, other triglycerides (i.e., TG 14:0-35:1, TG 14:0-36:1, TG 18:2-32:1, and TG 18:1-35:2), were all significantly higher in CM fed cows during HC4. Some of TG (i.e., TG 16:1-34:0) showed an interaction between treatment and parity, being increased in the CM diet only in primiparous cows and HC1. Of the PCs that were determined to be of interest by the VIP scoring, two showed a significant effect of interaction between additive and feeding phase ($P < 0.03$), but no effect of CM feeding (PC aa C 36:0 and PC aa C 38:1).

Conclusions: This study demonstrated changes in the metabolite profile of early lactation cows fed concentrate–rich diets. Feeding of CM increased plasma TG concentration of the cows, especially during the concentrate-rich feeding. This increase of certain TG classes with the feeding of CM in cows during early lactation may indicate enhancement of hepatic secretory capacity for TG in the systemic circulation with importance for lipid metabolism and transport, and thereby hepatic health and milk production.

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** Vetmeduni Wien Tierernährung und Funktionelle Pflanzenstoffe, Veterinärplatz1, 1210 Vienna, Austria, renee.petri@vetmeduni.ac.at*

75.

Effects of the addition of a mixture of mono- and diglycerides from butyric-, capric- and caprylic acid to compound feed on the success of experimental infection with *Salmonella Typhimurium* in weaned piglets

Effekte des Zusatzes einer Mischung von Mono- und Diglyceriden von Butter-, Caprin- und Caprylsäure zum Mischfutter auf den Erfolg einer experimentellen Infektion mit Salmonella Typhimurium bei Absetzferkeln

*Keller B., Buch J., Ader P., Visscher C. – Hanover / Lampertheim

A need to further reduce prevalence of *Salmonella* (*S.*) spp. on pig farms deserves particular attention due to zoonotic potential of these bacteria. In recent years, great efforts have been made. In this study the dietetic approach of a mono- and diglyceride mixture of butyric-, capric- and caprylic acid (mono-/diglyceride mixture), mainly containing 1-monobutyrine was tested *in vitro* and *in vivo* for potential effects on reducing *S.*.

Methods: For the *in vitro* study, freshly obtained, homogenized caecal chyme from piglets was supplemented with the mono-/diglyceride mixture (0, 0.5 or 1%), then inoculated with 1 x 10^7 cfu *S. Typhimurium* (*S.* T.)/sample. After 1 h, 4 h, 6 h and 24 h incubation time at 37 °C *S.* T. was determined qualitatively and quantitatively. In two different *in vivo* trials, in total 44 weaners were fed either conventional pelleted diet (control group, CG) or the same diet supplemented with 0.5% of the mono-/diglyceride mixture (Glyceride group, GG). For both trials, animals were housed in groups of 11 piglets. After seven days of adaptation to the diet in the first experiment only two weaners in CG and GG each were orally infected with 1x 10^7 cfu *S.* T.. In the second experiment each piglet was orally infected with 1x 10^7 cfu *S.* T. in the same way after natural exposure to *S.* Livingstone (*S.* L.). Depending on the distribution of the data in each experiment, results of qualitative and quantitative *S.* diagnostics were examined either for normally distributed data by t-test or by non-parametric Wilcoxon two-sample test for non-normally distributed data.

Results: *In vitro* experiments showed high significant *S.* T. reduction ($p < 0.01$) in the chyme inoculated with 1% of the mono-/diglyceride mixture compared to the control after 1 h incubation time as well as compared to the chyme inoculated with 0.5 % of that formulation. The difference in *S.* counts between the control chyme and the chyme supplemented with 0,5 % GG was not significant. In the first *in vivo* experiment, unexpected only a small number of animals was infected with rather low cell counts of *S.* T.. In the second experiment, there was no difference in prevalence of *S.* positive results (>90%), whereas quantitative analysis showed in some of the several samplings significantly higher *S.* counts in faeces of the experimental group (GG).

Conclusions: The *in vitro* experiments showed a reducing effect of the product on *S.* T. in the inoculated caecal chyme. This observation could not be confirmed *in vivo* by chosen set ups. Further investigations must clarify the cause of these discrepancies.

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* *University of Veterinary Medicine Hannover, Foundation Institute for Animal Nutrition, Bischofsholer Damm15, 30173 Hanover, Germany, birgit.keller@tiho-hannover.de*

76.

Impact of varying trypsin inhibitor activity in feed on chemical body composition of broiler chickens at the end of fattening

Einfluss einer variierenden Trypsininhibitoraktivität im Alleinfutter auf die chemische Zusammensetzung von Mastbroilern zum Mastende

*Kuenz S., Hoffmann D., Thurner S., Damme K., Windisch W., Brugger D. – Freising / Kitzingen

Clarke and Wiseman (1) state that trypsin inhibitor activity (TIA) should not exceed 4.0 mg/g in full-fat soybeans used for poultry feed. More recent investigations indicate reduced performance and amino acid digestibility even below that threshold (2, 3). There is so far no information available on the changes in chemical body composition. Thus, the present study investigated the effects of finely-graded differences in dietary TIA on chemical body composition of whole-body homogenates of broiler chicks at the end of fattening.

Methods: Raw material for this study consisted of two homogenous batches of soybeans (Sultana and Merlin) with a native TIA of 37.3 mg/g and 40.5 mg/g. Four processing techniques were used (thermal, hydrothermal, pressure and kilning) to create thirty-four soybean cake variants with a wide range of TIA (0.3 mg/g- 23.6 mg/g). These soy cake variants plus one variant solvent extracted soybean meal were included into a common grower and finisher diet for broiler chicks at fixed amounts (grower: 35%; finisher: 25%) and tested in a 35 d fattening experiment with 1,680 broiler chicks (grower phase: day 11 to 24; finisher phase day 25 to 35). At the end of the experiment, the birds were euthanized using electrical stunning and cervical dislocation. Two broilers per pen (420 in total) were randomly selected for determining whole body chemical composition. Feathers were removed from the bodies before freezing (-20°C) prior to homogenization. Homogenates were subjected to analysis of body water, crude protein (CP), crude fat (CL) and crude ash (CA). Also, the total feather weight as well as feather dry matter (DM) and CP were assayed. Statistical analyses included linear regression models (R 3.6.1). TIA was set as main factor; level of significance was determined as $\alpha = 0.05$.

Results: Water in whole body homogenates (%) increased significantly in a straight linear fashion with increasing dietary TIA (slope = +0.19, $P < 0.001$, $R^2 = 0.28$) while concentrations of CP (% DM) followed an indirect linear response (slope = -0.14 DM, $P < 0.01$, $R^2 = 0.28$). CA and CL concentrations were not affected. Total feather weight (g) decreased while feather DM (%) increased, in both cases in a significant straight linear fashion (slopes: -1.39 and 0.90, $P < 0.0001$ and $P = 0.02$, $R^2 = 0.69$ and 0.14). Feather CP concentrations (% DM) were not affected.

Conclusions: The data suggests that rising dietary TIA progressively induced undersupply of dietary protein and energy. Earlier studies indicated a linear decrease in zootechnical performance (2) (especially feed efficiency) and a linear decrease in prececal amino acid digestibility (3). In all these investigations, including the present one, the response was strictly linear even at low TIA levels. This indicates that the definition of an upper threshold for dietary TIA is not straightforward from a physiological point of view.

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

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* *Technische Universität München Lehrstuhl für Tierernährung, Liesel-Beckmann-Straße 2, 85354 Freising, Germany, sylvia.kuenz@wzw.tum.de*

77.

Effect of feeding industrial hemp to dairy cows on performance, health status and cannabinoid transfer into the milk

Einfluss der Verfütterung von Industriehanf an Milchkühe auf Leistung, Gesundheitsstatus und den Transfer von Cannabinoiden in die Milch

Wagner B., Gerletti P., Spolders M., Bernsmann T., Fürst P., Gusovius H.-J., Idler C., Lahrssen-Wiederholt M., *Pieper R. – Berlin / Münster / Potsdam

Industrial hemp is coming more and more into focus for a variety of uses. Hemp or by-products thereof may also be used as animal feed. The cultivation of industrial hemp in the EU is allowed only for varieties with a maximum delta-9-tetrahydrocannabinol (Δ9-THC) content of 0.2 %. Nevertheless there is concern about a possible transfer of cannabinoids into food of animal origin in case hemp or hemp by-products are used (1). Currently available data are limited and do not allow an appropriate risk assessment. The present experiment was conducted to quantify the transfer of cannabinoids into dairy cow´s milk as well as possible adverse effects on animals health.

Methods: A feeding trial was conducted with ten lactating Holstein Frisian dairy cows. All animals received a hemp-free diet for seven days before a period of feeding whole plant industrial hemp silage with low cannabinoid content (Δ9-THC: 58 mg/kg DM, cannabidiol (CBD): 805 mg/kg DM) for seven days (adaption period). Subsequently, animals were separated into two groups receiving industrial hemp silage produced from leaves, flowers and seeds with higher cannabinoid content (exposure period; Δ9-THC: 1255 mg/kg DM, CBD: 8304 mg/kg DM). Cows belonging to group 1 received 0.84 kg DM/day, while groups 2 cows received 1.68 kg DM/day, to determine dose-dependent effects on the transfer of cannabinoids into milk, cow´s performance and health status. Finally, both groups were fed with hemp-free diets for another eight days (post-exposure period). Milk samples were taken regularly and animal health parameters and performance (feed intake, milk yield, respiratory, heart, and rumination rates) were recorded. The concentration of seven different cannabinoids and two Δ9-THC metabolites was measured in milk, blood, urine and faeces using a new approach based on HPLC-MS/MS. This allowed the analytical differentiation between Δ9-THC and its acid precursor Δ9-THCA, crucial to the correctness of the investigation. Data analysis was performed using SPSS and a toxicokinetic model was developed using Python3.

Results: During the first two weeks animals showed no clinical symptoms and low cannabinoids levels were detected in milk by the end of the adaptation period. During the exposure period the average intake of Δ9-THC was approximately 1000 mg/(animal*day) for group 1 and 1700 mg/(animal*day) for group 2, corresponding to an average daily dose of 1,6 mg (group 1) and 2,8 mg (group 2) Δ9-THC per kg body weight. The average CBD intake was approximately 6900 (group 1) and 11000 mg/(animal*day) (group 2), corresponding to an average daily dose of 10,7 mg (group 1) and 18,4 mg (group 2) CBD per kg body weight. A significant ($p<0.001$) decline in respiratory and heart rate was observed during this period. Concomitantly feed intake was significantly reduced, resulting in a decreased milk yield ($p<0.01$). The cannabinoid concentration in milk reached nearly 0.5 mg/kg for Δ9-THC and over 2 mg/kg for CBD. The other cannabinoids were detected as well. The apparent average transfer rate for Δ9-THC was approximately 0.2 % and 0.1 % for CBD.

Conclusions: Feeding silage derived from industrial hemp with in Δ9-THC content close to the maximum permitted levels of 0.2 % and even higher CBD content to dairy cows can result in considerable levels in the milk. Moreover animal performance and health parameters are significantly affected. A cannabinoid transfer from feed into milk is possible depending on hemp variety and plant parts used. The relevance of the use of hemp silage for practical feeding of dairy cows needs further clarification. Moreover, the relevance of the results for consumer safety and animal welfare requires further investigation. The data may help to derive maximum dietary inclusion levels for industrial hemp in dairy cows' diet.

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* *German Federal Institute for Risk Assessment, Berlin, Germany, robert.pieper@bfr.bund.de*

78.

Effects of glyphosate residues and different concentrate feed proportions in dairy cow rations on hepatic gene expression, liver histology and activity of enzymes indicative for liver damage

Einflüsse von Glyphosatrückständen und variierenden Kraftfutteranteilen in Milchkuhrationen auf die Genexpression, Histologie und die Aktivität von Indikatorenzymen für Schädigungen der Leber

*Heymann A.-K., Schnabel K., Billenkamp F., Bühler S., Frahm J., Kersten S., von Soosten D., Meyer U., Oster M., Teifke J. P., Dänicke S. – Braunschweig / Dummerstorf / Insel Riems

Glyphosate (GLY) is one of the most used broadband herbicides worldwide and possible side-effects are controversially discussed in public, especially in the context of animal health. Due to its wide use in agriculture, GLY residues are found in dairy cows' diets (1). Accordingly, the cow has to cope with these xenobiotic residues. The present study investigated worst-case conditions of GLY-contaminated feed due to a pre-harvest treatment of plants according to regulation (EC) No. 396/2005. In the view that the liver is the primary target of xenobiotics the aim was to analyze GLY effects on liver gene expression and histology as well as on the activity of enzymes indicative for liver damage in the context of different concentrate feed proportions in lactating dairy cows' rations.

Methods: A 17-week feeding trial was conducted (2). For adaption, 61 German Holstein cows in lactation received a total mixed ration (TMR) containing 40% concentrate based on dry matter (DM, week 0). After this period, the cows were assigned to groups fed with either GLY-contaminated TMR (GLY, GLY intake >73mg/cow/d) or control TMR (CON, GLY intake <1mg/cow/d). Both groups were further split into subgroups with high concentrate (HC,60% based on DM) or low concentrate (LC,30% based on DM) proportions in TMR. For RNA sequencing, total RNA was isolated from liver biopsy samples of 31 cows (7-8 cows/group) collected in week 16 and used for a 2x101 bp paired end read Illumina HiSeq sequencing approach. Differentially expressed genes (DEGs) were quantified and functionally characterized using the R package DESeq2 and the Web-based software tools DAVID and BlastKOALA. Furthermore, histopathological analysis was performed on HE-stained liver samples of 31 cows taken in week 0,8 and 16. Blood samples were collected in week 0,4,8,12 and 16 for determination of aspartate aminotransferase (AST), γ-glytamyltransferase (GGT) and glutamate dehydrogenase (GLDH) activities in serum using a photometric approach (Eurolyser). SAS 9.4 (Proc MIXED) was used for statistical analyses.

Results: Gene expression analysis resulted in 167 concentrate-feed proportion (CFP) dependent DEGs ($-0.61 < \log2$ fold change (lfc)< 0.56, $p < 0.05$, false discovery rate (FDR)$< 10\%$), while only seven genes showed different transcript abundances in GLY groups compared to CON groups ($-0.38 < lfc < 0.50$, $p < 0.05$, $FDR < 10\%$). Functional clustering of the CFP-responsive DEGs showed a significant enrichment of 21 DEGs in four KEGG pathways. In detail, seven DEGs were enriched in both carbon metabolism and chemical carcinogenesis, while six DEGs were assigned to the complement and coagulation cascade and to the metabolism of xenobiotics by cytochrome P450, respectively. Furthermore, overlaps of DEG assignment to chemical carcinogenesis and metabolism of xenobiotics by cytochrome P450 occurred. Contrary, no significant enriched GLY-responsive KEGG pathways were found. However, the seven putatively GLY-responsive DEGs included genes encoding two calcium-dependent proteins, a cation channel, a negative regulator protein of a receptor as well as an adaptor protein. Histopathological evaluation of liver samples as well as AST, GGT and GLDH activities in serum showed significant CFP effects, while no significant GLY effects were observable. Activities of AST, GGT and GLDH as well as histological scores were higher in HC groups than in LC groups. Last mentioned might be mostly due to an increase of apoptotic and necrotic cells, portal inflammation and sinusoidal dilatation. The observations of significant CFP effects and missing GLY effects are in agreement with previous results of this study, where CFP and GLY influences on parameters like body weight or milk yield were determined (2).

Conclusions: CFP influences the hepatic gene expression, liver histological parameters and activities of AST, GGT and GLDH, while GLY only seems to influence gene expression slightly. Gene expression results were validated by qRT-PCR.

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* *Friedrich-Löffler-Institut Institute of Animal Nutrition, Braunschweig, Germany, ann-katrin.heymann@fli.de*

79.

Hypoglycin A and methylenecyclopropylglycine intoxication after the ingestion of sycamore maple seeds in 3 Père David's deers (*Elaphurus davidianus*) with Atypical Myopathy

Vergiftung durch Hypoglycin A und Methylencyclopropylglycin nach Aufnahme von Bergahornsamen bei 3 Davidshirschen (Elaphurus davidianus) mit Atypischer Myopathie

*Bochnia M., Ziemssen E., Sander J., Stief B., Zeyner A. – Halle (Saale) / Dresden / Hanover

The intake of hypoglycin A (HGA) and methylenecyclopropylglycine (MCPG) from seeds/seedlings of Sycamore maple (SM, *Acer pseudoplatanus)* causes Atypical Myopathy (AM) in horses, which is characterized by colic, sweating, trembling, myoglobinuria and death (1). The toxicity of HGA and MCPG was first described in humans after ingestion of soap-berry fruits (2). Poisoning requires a conversion of these compounds into CoA derivatives (MCPA-CoA, MCPF-CoA), which inhibit acyl-CoA-dehydrogenases and –dehydratases interrupting the energy production by ß-oxidation of fatty acids. AM in wild ruminants was unknown until several fatalities in deers (*E. davidianus*) were related to HGA ingested with SM seeds (3). Aim of the study was to show that besides HGA, MCPG is also involved in AM in deers.

Methods: In Sep 2018, three deers from the zoo Dresden (M1-3; 7-11 years, 2 female, 1 male, good BCS) developed AM with trembling, muscle pain, salivation, dysphagia and recumbency after the ingestion of SM seeds and leaves. M1 died, M2 and M3 were euthanized. M1 and M2 were dissected. Seed and leave extracts were analyzed for HGA and MCPG [1]. Serum, urine and methanol extracts from liver, kidney, rumen digesta and faeces from M2 and M3 were analyzed by UPLC-MS/MS [1] for HGA, MCPG and for conjugates of carnitine (C) and glycine (G): MCPA-G, MCPA-C, MCPF-G, MCPF-C, butyryl-C and isobutyryl-C. Creatinine kinase (CK) and lactate dehydrogenase (LDH) were analyzed in blood serum (photometrically).

Results: HGA [nmol/L] varied from 204 – 93.228 in seeds and reached 38.750 in leaves. MCPG [nmol/L] ranged from 5 – 96.496 in seeds and 3 – 193 in leaves. CK (U/L: M1 312.180; M2 337.808; reference (ref) cattle < 150) and LDH (U/L: M1 16.169; M2 15.038, ref < 150) were extremely elevated, indicating severe muscle cell damage. HGA ([nmol/L] M2 482; M3 456; control horses (CO) < 0.8), but not MCPG was high in serum. Both were high in rumen contents (HGA, M2 1.728, M3 1.753; MCPG, M2 924, M3 864), liver of M3 (1.181) and faeces of M2 and M3 (HGA, 309 and 2.024; MCPG, M3 1.433), but not in kidney. Metabolites [nmol/L] from HGA and MCPG were high in serum (MCPA-G, M2 738, CO < 1.9; MCPF-C, M2 18.349, M3 9.615; CO < 0.8), urine (MCPA-G, M2 16.785, M3 4.577; MCPF-G, M2 7.546, M3 1.827, CO < 0.8; MCPF-C, M2 21.257, M3 14.054) and liver (MCPA-G, M2 3.603, M3 1.147; MCPF-C, M2 4.661, M3 1.497), partly in kidney, but not in faeces. Butyryl-C ([µmol/L] serum, M2 294, M3 165; urine, M2 254, M3 186; CO < 0.2) and isobutyryl-C (urine, M2 139, M3 82; CO < 0.5) were increased caused by inhibition of ß-oxidation. Sections (M1/2) show a multifocally located acute high-grade monophasic myelonecrosis.

Conclusions: The results show thatMCPG is also involved in AM in deers. The low MCPG concentration in serum may indicate that MCPG is metabolized faster than HGA and therefore appears, highly concentrated, in the form of derivates. HGA and MCPG in seeds and leaves of SM present a major hazard also for zoo animals.

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* *Martin Luther University Halle-Wittenberg, Halle (Saale), Germany, mandy.bochnia@landw.uni-halle.de*

80.

Effects of faba beans with different concentrations of vicine and convicine in the diet on the performance and egg quality of laying hens

Einfluss von Ackerbohnen mit einer unterschiedlichen Konzentration an Vicin/Convicin im Futter auf die Entwicklung der Leistungen von Hennen und der Eiqualität

*Halle I. – Braunschweig

In Germany faba beans are cultivated as high protein seeds on 55,300 hectares. This is a marginal cultivation in comparison to that of rape seed which has to be justified by its vulnerability towards water shortage during cultivation and to anti-nutritive seed substances, like tannine, vicine and convicine. In 2015, the BLE initiated the project "Breeding and agronomy of new, vicine poor field beans and use as a local proteins feed" to minimise these disadvantages and to enhance the use of the field bean as a feedstuff, The aim of this project is to cultivate a winter field bean with a higher yield potential because of less dry stress and its use in the feed also of sensitive animal species, like poultry.

The following study was carried out to verify the influence of increasing proportions of faba beans with high and low vicine/convicine (V/C) – concentrations in diets on the performance of laying hens.

Methods: 400 hens (Lohmann Brown) were randomly divided into 5 groups. The hens were kept in pens (20 hens per pen) with 4 pens per group. The study commenced when the hens were 21 weeks old and continued until the end of the 6th laying month (168 days). In the treatments faba beans concentration (0/15/30%) was increased and soybean meal decreased per kg feed. The influence of the new cultivated poor vicine/convicine (0.045% dry matter) winter field bean was compared with a field bean which was rich in vicine/convicine (0.760% dry matter). Number of eggs laid per pen was recorded daily and the feed consumption monthly. Each month the collected eggs were weighed four times within two weeks. In the 6th laying month, all eggs laid per pen over 3 consecutive days were collected to measure egg composition. Data of laying performance 2 to 6 were analysed with ANOVA (SAS) and the Student-Newman-Keuls-Test ($p<0.05$).

Results: In the 1st laying month the daily feed intake (97 - 105 g/hen/day) of the hens and also the laying intensity (22 - 30%) and the egg weight (52 - 56 g/egg) were low in all groups, that's why the results of the laying performances of laying month 2 to 6 were statistical analysed. In these five laying month the laying intensity (83.5 - 88.8%) was not significantly different between groups. The daily feed intake (117 g/hen), egg weight (60.9 g/egg) and egg mass production (51.0 g/hen/day) of hens from the 30% V/C rich field beans were significantly ($P<0.001$) decreased compared to the other groups (120 - 124 g feed intake/hen/day; 64.2 - 65.6 g egg weight; 56.1 - 57.9 g egg mass production/hen/day). In the groups with 15% and 30% V/C rich field beans the feed conversion ratio was 2.20 kg/kg and 2.33 kg/kg compared to control 2.13 kg/kg and 2.16/2.15 kg/kg in groups with 15/30% V/C poor field beans. In the 6th laying month the egg composition showed a significantly ($P<0.001$) lower egg weight (63.2 g/egg), a higher percentage of egg yolk (25.8%) and a reduced egg shell breaking strength (46.8 N) of hens of the 30% V/C rich field beans group compared to the 30% V/C poor field beans group (64.9 g weight; 25.2% egg yolk; 52.7 N egg shell breaking strength). The body weight of the hens was not significantly different between the groups at the start and at the end of the study.

Conclusions: The results showed that the content of 30% V/C poor winter field beans in the diet did not have negative effects on feed intake, laying performance, egg weight, egg composition, egg shell breaking strength and feed conversion of laying hens.

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* *Federal Research Institute for Animal Health (FLI) Institute of Animal Nutrition, Bundesallee 37, 38116 Braunschweig, Germany, ingrid.halle@fli.de*

81.

Influence of accidental feeding of the coccidiostat Nicarbacin on health and performance of laying hens – a case report

Einfluss der versehentlichen Verfütterung des Kokzidiostatikums Nicarbazin auf Gesundheit und Leistung von Legehennen – ein Fallbericht

Rosenthal F., Spolders M., *Pieper R. – Berlin

Nicarbacin is currently authorized in the EU as a feed additive in controlling coccidiosis in chickens for fattening (1). Nicarbacin may have negative effects on animal health when added non-intentional to the feed of non-target animal species such as horses, turkeys or rabbits (EFSA, 2010). However, negative effects of Nicarbacin on health and performance of laying hens were yet not addressed.

Methods: In a breeding hen farm (Lohmann brown-classic) producing eggs for hatching, a coccidiostat-containing premix (containing Nicarbacin/Narasin, 1:1 w/w) for fattening chicken was accidentally mixed into the feed at 0.5% of the diet instead of a coccidiostat-free premix. As a consequence, the laying performance (number of eggs and egg weight) declined rapidly about 30% and mortality rate increased within a few days after feeding of the new feed. Egg shells decolorized showing all stages between pale white and dark brown. Approximately >25,000 eggs for hatching were discarded as hatching performance severely declined. A follow up on the case included three main steps: I) an analysis of nutrient composition of diets with correct premix and the non-intentionally used premix to clarify whether (micro-)nutrient supply could explain the observed effects, II) a detailed analysis of data on laying performance and mortality in conjunction with water use (as indirect indicator for heat stress) on the farm, and analysis of data on hatching performance, III) a literature review regarding the putative link between coccidiostats (and specifically Nicarbacin and Narasin) and the observed effects in this case.

Results: The final concentration in the feed was 124 mg/kg Nicarbacin and 120 mg/kg Narasin, respectively whereas the maximum allowance in diet for fattening chickens is 50 mg/kg diet (1), respectively. Moreover, the maximum non-intended carry-over into feed for laying hens is 1%. A detailed (micro-)nutrient analysis is ongoing. It cannot be excluded that a deficiency in micronutrients and vitamins could have impaired animal responsiveness against the coccidiostat-induced effects. Data from the affected barns revealed that the decline in laying performance and increased mortality occured rapidly after feeding of the new diet (onset after 2 days). Interestingly, the feed was only offered up to 50 h in the most affected barn but symptoms persisted for several days thereafter. Barn data also revealed a higher water intake after feeding the coccidiostat-containing diets suggesting that these animals experienced increased heat stress. It has been shown in several studies that Nicarbacin can lead to increased mortality under heat stress. This effect may be even more pronounced in older animals such as breeding hens. A combination with Narasin reduces the required amount of Nicarbacin to act against coccidia thereby reducing heat stress-induced health effects. However, the accidentially used amount of Nicarbacin in the diet of the present case was likely too high. A recent study has shown that Nicarbacin inhibits delta-aminolevulinate synthase 1 (ALAS 1), the rate-limiting enzyme for Protoporphyrin IX (PP IX) and heme production (3). Moreover, Narasin has been associated with reduced egg weight. Together, this could help to explain the observed effects of decolorized egg shells, reduced laying and hatching performance, and increased mortality.

Conclusions: The case reveals clearly that a non-intended use of the coccidiostat Nicarbacin in the diet for laying hens could result in dramatic effects on egg shell color, laying performance and mortality within a few days. It once again highlights the necessity of handling coccidiostats with care to avoid (cross-)contamination of feed for non-target animal species.

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* *German Federal Institute for Risk Assessment, Berlin, Germany, robert.pieper@bfr.bund.de*

82.

Detection of antibiotic residues in colostrum of dairy cows

Nachweis von Antibiotika-Rückständen in Kolostrum von Milchrindern

Reulmann S., Hayer J. J., Heinemann C., *Steinhoff-Wagner J. – Bonn

Feeding colostrum of good quality is crucial for the survival of neonatal mammalians. For years, colostrum quality has predominately been determined by protein content, especially immunoglobulins. With raising production standards, sustainability aspects such as reducing the spread of antibiotic residues or the development of antibiotic resistant bacteria, become more important. The aim of this pilot study was to address methodical aspects related to the determination of antibiotic residues in colostrum.

Methods: Colostrum samples of 55 dairy cows from 7 dairy farms were collected immediately after birth, within 12 hours and 3 days after birth. From each cow information on the drying-off duration, the method and agents used for sealing as well as the number of calvings were recorded. All samples were analyzed for dry matter, density (Digital Brix Refractometer, Milwaukee Instruments, USA), pH (testo, Lenzkirch, Germany) and for antibiotic residues by a commercially available version of the brilliant black reduction test (MILKU Tierhygiene, Germany). Additionally to the listed three-point scale based on a change in color (no, moderate or strong), automatic read-out methods via pH and color values (blue, green, red) were evaluated. Data were analyzed with Spearman rank correlations and linear models with post hoc Tukey for farm, sealing method, antibiotic agent and drying-off duration with SAS 9.4 (2016).

Results: As is known for mature milk, pH of colostrum from day 3 is dependent on farm specific factors, whereas farm-specific differences in the denser early colostrum samples were absent. Dry matter correlated with digital density ($R=0.87$; $p<0.001$; $n=96$). However, in some cases automatic calculations seem to lead to an underestimation of the digitally determined density in comparison to the dry matter. In total, 13 samples were found strongly positive for antibiotic residues. Additionally, 6 samples showed a moderate change in color. The positive samples were distributed evenly over the first 3 days after calving (7 after birth, 6 within 12 hours after birth and 6 on day 3). Positive test results expressed with a three-point scale as well as automatically determined by pH of the test were positively correlated with the drying-off duration ($-0.3>R>-0.4$; $p<0.01$; $n>95$, respectively) and were influenced by the initial pH of the colostrum. Particularly, on farms with relatively short drying-off duration (5 weeks) and relatively long withdrawal times of milk for the used antibiotic agents (> 6.5 weeks) showed a high proportion of positive colostrum samples (9 of 17).

Conclusions: Antibiotic residues in bovine colostrum might be a serious risk for newborn calves. Measuring the pH of the brilliant black reduction test seems to be very useful for rapid analysis of antibiotic residues. For a systematic analysis of risk factors, using the brilliant black reduction test might be a cost-efficient way to collect a comprehensive data set; however, results might be corrected by the initial pH of the colostrum.

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* *Rheinische Friedrich-Wilhelms-Universität Bonn Insitut für Tierwissenschaften, Katzenburgweg 7-9, 53115 Bonn, Germany, jste@itw.uni-bonn.de*

83.

Applying *in vitro* batch cultivation to preliminary investigate pyrrolizidine alkaloids transformation in ruminal inoculum from dairy cows

Anwendung einer in vitro Batch Kultivierung zur Untersuchung des Abbaus von Pyrrolizidinalkaloiden in Panseninokulum von Milchkühen

*Tänzer J., These A., Spolders M., Pieper R., Klevenhusen F. – Berlin

Pyrrolizidine alkaloids (PA) are secondary plant metabolites which mainly appear in plants of the families *Asteraceae*, *Fabaceae* and *Boraginaceae*. They can occur as tertiary amines (free base) and their *N*-Oxides, respectively. PA are known to cause hepatotoxic effects in humans and ruminants (1). The feeding of contaminated forage or the accidental grazing of PA plants has not only an impact on the health of ruminants but can also pose a risk to humans through transfer into products of animal origin. Knowledge on the fate of PA in ruminants will help to assess these risks. The microbial ecosystem in the rumen may have an impact on PA biotransformation prior to absorption and further metabolism. Here, we examined the fate of selected PA in the ruminal inoculum of Holstein dairy cows using an *in vitro* batch culture approach.

Methods: Three independent batch culture runs were conducted. Each run consisted of three independent experiments. Each experiment was done with ruminal inoculum from a different lactating cow fed maize-silage based diet. The inoculums (liquid and solid parts in equal amounts) were buffered (1:4; w:w) and an aqueous extract of *Senecio Jacobeae* (containing 70 – 27884 ng/mL individual free base and *N*-Oxide PA) was added. These final batch culture samples had individual free base concentrations from 3.3 to 30.0 ng/mL and individual *N*-Oxide concentrations from 14.9 to 1367.8 ng/mL. The samples where incubated in duplicate anaerobically for 20 h at 39 °C. After 0, 1, 2, 4, 6, 8 and 20 hours of incubation, pH-values and redox potentials were measured and biological reactions were stopped by addition of 0.05 M sulfuric acid. After centrifugation, PA were directly analysed by mass spectrometry in MRM mode (Agilent 6495). Each batch culture run consisted of 3×14 samples. Results were validated against a negative control with autoclaved ruminal inoculum. Statistical analysis was performed with SAS (Version 9.4).

Results: Differences in pH-values were observed between individual cows' inocula resulting in slightly different pH-values during batch cultivation (cow effect $P<0.001$). All individual *N*-oxide forms disappeared rapidly and their concentrations decreased below the limit of detection within one hour of incubation. Meanwhile, the concentration of the corresponding free base form increased within one hour suggesting an initial transformation of the *N*-Oxide into their respective free base form. After an incubation period of multiple hours, most of the measured free base forms disappeared and their concentrations decreased below the limit of detection, suggesting either a degradation or biotransformation into yet unknown metabolites. All PA showed a variation of their concentration with the duration of incubation ($P<0.01$). A cow effect was observed for several PA indicating differences in microbial activity.

Conclusions: The investigations revealed that 1) especially *N*-oxide forms of PA are rapidly degraded by the ruminal microbes, 2) the free base is likely the first metabolite formed from the *N*-oxide and 3) after multiple hours also the free base forms are degraded extensively. Further research is needed to determine the metabolites of ruminal PA biotransformation and to gain knowledge on the absorption from the ruminal/intestinal lumen.

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* *German Federal Institute for Risk Assessment 8, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany, julian.taenzer@bfr.bund.de*

84.

Rhizoctonia sp. on a weed (*Cerastium holosteoides*) causing hypersalivation in horses – a case report

Rhizoctonia sp. auf einem Kraut (Cerastium holosteoides) als Auslöser von Hypersalivation bei Pferden – ein Fallbericht

*Böswald L. F., Schwaiger K., Kienzle E. – Oberschleißheim

An increased number of outbreaks of hypersalivation in horses was observed over the past years. Slaframine intoxication is a known cause for this symptom in several species ("slobbers syndrome"). According to literature (1), the mycotoxin slaframine is produced by *Rhizoctonia leguminicola*, the fungus that causes black patch disease of clover or other legumes (2). In this case report, slaframine intoxication was suspected in horses.

Case history: Four horses in a privately owned stable showed hypersalivation after grazing on the pastures. The amount of serous saliva produced was extremely high, so that small puddles formed on the ground where the horses stood. No other signs of disease or infection were observed. Hypersalivation receeded after keeping them indoors without access to the pastures and feeding hay only for several weeks. The hay fed in the stable was of average quality and did not show conspicuous details. There was not a lot of clover in hay and forage and it did not show signs of black patch disease. A visit to the pastures revealed that part of the grasses had black spots on the leaves and that several weeds were growing among the grass, some of which showed signs of plant disease. The plants with abnormal findings like yellowish leaves or dark spots were sampled individually and semiquantitative mycological analysis was performed. On the common mouse-ear chickweed, *Cerastium holosteoides*, the fungus *Rhizoctonia solani* was identified. The recommendation was to close off the pastures and keep the horses with limited access to a paddock area without fresh forage. All *C. holosteoides* plants should be weeded. After removal of this plant from the pastures, the horses were allowed on the pastures again for increasing time spans. No further hypersalivation occurred.

Discussion: The massive hypersalivation in several horses without other clinical abnormalities was typical of the slobbers syndrome. Because of the occurrence only during/after grazing on the pastures, a connection between the symptoms and plants on the pastures could be drawn. The infestation of *C. holosteoides* with *R. solani* is remarkable, because this fungus if from the same family as *R. legumincola*, the known agent producing slaframine. The cessation of hypersalivation after removal of the infested plants is also an indicator, that *Rhizoctonia* sp. on *C. holosteoides* had been the cause. In cases of hypersalivation, mycological analysis should always be performed to identify potential toxin producing agents. If possible, mycotoxin assays should also be performed from the isolated cultures.

Conclusions: *Rhizoctonia* sp. on plants other than legumes can be associated with hypersalivation in horses. Possibly, these new combinations of fungal species and plants may be due to changes in climate.

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* *Ludwig-Maximilians-Universität München Chair of Animal Nutrition and Dietetics, Oberschleißheim, Germany, linda.boeswald@lmu.de*

85.

Do dietary phytase and myo-inositol supplementations differently affect the plasma metabolome profile of broiler chickens?

Beeinflusst die Supplementierung mit Phytase und Myo-Inositol das Plasmametaboliten-Profil von Broilern unterschiedlich?

*Gonzalez-Uarquin F., Kenez A., Rodehutscord M., Huber K. – Stuttgart / Hong Kong

Myo-inositol is an important constituent of animal cells, where it has shown to be crucial for several metabolic and regulatory processes (1). Myo-inositol is synthesized endogenously but also absorbed from dietary sources. One of the main sources of dietary myo-inositol is phytic acid, which is partially hydrolyzed by phytases and phosphatases to release myo-inositol, phosphate, and other nutrients in the intestine (2). Metabolic effects of myo-inositol in relation to phytase supplementation are unknown in chickens, so that this research aimed to assess the differential effects of myo-inositol and phytase supplementations on the blood plasma metabolome profile of broiler chickens.

Methods: Broilers were provided a nutrient adequate basal diet (Control), a basal diet plus 3.5 g myo-inositol (MI), and a basal diet plus 500 (PHY500), 1500 (PHY1500), or 3000 (PHY3000) units of phytase per kg feed. All broilers were group-housed in floor pens (8 pens per diet) and diets were provided until 22 days of age (3). Blood plasma samples were collected from one bird per pen, i.e. 8 replicated measurements per diet were made. The targeted AbsoluteIDQ p180 Kit (Biocrates Life Sciences AG) was utilized for metabolite quantitation. To compare Control and MI groups as well as the effects of myo-inositol supplementation on the metabolite profile, Student's t-test and Volcano Plot were used, respectively. For comparison between Control and phytase supplemented groups and metabolite profile identification, one-way ANOVA followed by Tukey's Honest Significant Difference test and Euclidean heat-map were used.

Results: Body weight was not affected by myo-inositol or phytase supplementation. Myo-inositol in plasma increased after supplementation of myo-inositol ($P<0.001$) and 500 or 1500 units/kg of phytase ($P=0.012$). The targeted metabolomics approach indicated that myo-inositol supplementation increased plasma concentrations of dopamine ($P=0.028$) and serotonin ($P=0.002$) in comparison to Control. With regard to the different levels of phytase supplementation, significant variations in 14 acyl-carnitines, 25 phosphatidylcholines, 6 lysophosphatidycholines, 6 sphingomyelins, and 4 biogenic amines were observed. Metabolites from the mentioned groups, with the exception of c4-OH-Proline ($P=0.006$) and histamine ($P=0.049$), decreased right after all levels of phytase supplementation ($P<0.05$).

Conclusions: The myo-inositol supplement can affect the dopamine-serotonin axis in broiler chickens. Although phytase supplements increased plasma myo-inositol concentrations, metabolome profile responses were different from pure myo-inositol supplements. This indicates that the metabolome profile was affected by other degradation products such as phosphate, other minerals, or inositol phosphates. The present findings could be indicative for further research related to the relationships between phytase and myo-inositol supplements and broiler health and behavior.

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* *University of Hohenheim Institute of Animal Science (460), Garbenstrasse 17, 70599 Stuttgart, Germany, fernando.gonzalez@uni-hohenheim.de*

86.

Growth performance, carbohydrate and fat metabolism and specific target gene expression in female fattening pigs after feeding phytogenics – pilot study

Wachstumsleistung, Kohlenhydrat- und Fettstoffwechsel und Expression spezifischer Zielgene bei weiblichen Mastschweinen nach Fütterung phytogener Zusätze - Pilotstudie

*Bochnia M., Aumiller T., Mueller A., Wensch-Dorendorf M., Bulang M., van der Klis J. D., Zeyner A. – Halle (Saale) / Steyregg

Selected phytogenics from *Fabaceae* seeds (FS) (1), *Apiacaeae* roots (AR) (2) and *Moringaceae* leaves (ML) have been reported to mitigate diabetes in animals and humans (3). In the according literature, mainly diabetic rodents were used as model animals. We aimed to investigate the effects of the mentioned phytogenics (single or in combination) on growth performance, carbohydrate and fat metabolism and expression of specific target genes in healthy female fattening pigs.

Methods: In each of two separate rounds, 48 female fattening pigs ([German Large White x German Landrace] x Duroc) were allocated in pens (n = 3/pen) and fed *ad libitum* during the grower (GRO) and finisher (FIN) period (35 - 90 kg). A basal adaption diet without additives was fed for 14d, then pigs were randomly allocated to the 4 treatments (T1 - T4) with each 0.2% of phytogenic preparation: T1, basal diet + wheat bran as negative control; T2, basal diet + FS and FS extracts; T3, diet T2 + ML; T4, diet T3 + AR. Feed intake and body weight (bwt) of pigs (start, end GRO, end FIN) were recorded. At the end of FIN, blood for determination of glucose, fructosamine, insulin, triglycerides, free fatty acids, cholesterol, HDL and LDL, and neck fat from the carcass for gene expression analysis of selected genes (IRS1, PDPK1, PPAR gamma, S6KB1) by qPCR were sampled in the slaughterhouse. The carcass lean meat proportion (LM) was specified by a skilled butcher. SAS 9.4 was used for statistics.

Results: In round 1 one pen was excluded from analysis since one pig suffered from bronchitis. All diets were accepted well by the pigs, with no effect on feed intake ($P > 0.05$). During the GRO period weight gain (kg/pig) was higher in T2 (28.2) and T4 (28.4) than in T1 (25.6; $P < 0.05$), but not in T3 (26.5; $P > 0.05$). This effect was inverted during the FIN period (T1 38.8 *vs* T2 37.1, T3 37.8 and T4 37.2; $P > 0.05$). During the GRO period, feed conversion ratio (kg feed/kg bwt gain) differed numerically between groups (T1 1.69, T2 1.49, T3 1.63, T4 1.47; $P > 0.05$). Similar observations were also made in the FIN period (T1 2.01, T2 2.14, T3 2.14, T4 2.06; $P > 0.05$). Serum parameters of glucose and lipid metabolism were not influenced by any treatment ($P > 0.05$). LM quality was overall high (68/96 pigs: > 60% LM) not influenced by the diet ($P > 0.05$). Compared to the qPCR dcQ-values in T1 (IRS, 7.49; PDPK1, 6.89; PPAR gamma, 5.82), fat tissue gene expression showed an up-regulation for IRS1 (7.98; $P < 0.05$) and PDPK1 (7.23; $P < 0.1$) in T4, and for PPAR gamma in T3 (6.38; $P < 0.05$) and T2 (6.29; $P < 0.01$).

Conclusions: Phytogenics may influence weight gain in the early fattening period and the expression of specific target genes which are involved in glucose-, insulin- and lipid-metabolism, with no consequences on serum glucose, insulin and triglycerides in healthy female pigs. Nevertheless, transport and stress before slaughtering might have had a higher impact on individual blood parameters than phytogenics. Further studies should use blood samples taken at the home stable.

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* *Martin Luther University Halle-Wittenberg, Halle (Saale), Germany, mandy.bochnia@landw.uni-halle.de*

87.

Influence of intensive feeding on insulin signalling in tissues of Holstein fattening bulls

Einfluss einer intensiven Fütterung auf den Insulinsignalweg bei Holstein Mastbullen

*Bäßler S. C., Koch C., Scheu T., Dänicke S., Meyer U., Huber K. – Stuttgart / Münchweiler an der Alsenz / Braunschweig

Intensive fattening, based on high amounts of concentrate in the ration, is a common practice to fatten bulls. This feeding can lead to subacute ruminal acidosis and an increase of microbial endotoxins in rumen and blood, consequently enhancing inflammatory mediators (1). Furthermore, easily digestible grain-based and starch-rich nutritional regimen in other species, such as humans or horses, leads to changes in metabolism and health, for example insulin dysregulation and metabolic syndrome (2, 3). Insulin dysregulation could be based on variations in tissue insulin signalling causing insulin resistance. Hypothetically, high intensity of fattening may decrease insulin signalling capacity in bulls. Therefore, the aim of this study was to determine the differences in protein expression and extent of phosphorylation of key components in insulin signalling in liver, muscle and retroperitoneal adipose tissue at the end of the fattening period.

Methods: Holstein bulls intended for beef production were randomly assigned to an intensive (n=15) or a moderate nutritional regimen (n=15). Diets were based on corn- and grass-silage and the intensive group received a surplus of 6 kg concentrate/day/animal for the final 8 months of the fattening period. Bulls were slaughtered at the age of 20 months, about 4 h after the last feed intake. Blood plasma, liver, skeletal muscle and retroperitoneal adipose tissue samples were collected. Glucose (Cobas c311 Analyzer, ROCHE, Mannheim, Germany) and Insulin (ELISA; Mercodia AB, Uppsala, Sweden) were measured in the plasma. Protein expression and extent of phosphorylation of insulin receptor (INSR; at Tyr1150), protein kinase B (AKT; at Ser473), AMP-activated kinase (AMPK; at Thr172) and mammalian target of rapamycin (mTOR; at Ser2448) were semiquantified by Western blot. Values under or above mean ± 2xSD were excluded as outliers. Results between feeding groups were compared by unpaired student's t-test. Level of significance was set at $P < 0.05$.

Results: Final body weight of the bulls was 807 ± 9 kg and 712 ± 12 kg (mean ± SEM) in the intensively fed and control groups, respectively. Plasma insulin concentration, protein expression and extent of phosphorylation in the three tissues were affected by the diet. In the liver expression of AKT ($p<0.0001$), mTOR ($p=0.005$) and AMPK ($p=0.0013$) was higher in the intensively fed group, also extent of phosphorylation of mTOR ($p=0.0085$) and AMPK ($p=0.0387$). The ratio of phosphorylated/non-phosphorylated protein in the liver was higher for pAMPK/AMPK ($p=0.048$), but was lower for pAKT/AKT ($p=0.0056$). In muscle and adipose tissue, expression of INSR ($p=0.0252$/ $p=0.0004$) was lower in intensively fed bulls and in adipose tissue expression of AKT ($p=0.035$) was lower, too. In muscle pAMPK/ AMPK ratio ($p=0.0025$) was lower in intensively fed group. In all three tissues extent of phosphorylation of INSR were under the limit of detection, as well as of mTOR in muscle. Concentration of insulin in the plasma was greater ($p<0.0001$) in intensively fed bulls; however, this did not influence glucose concentration in plasma.

Conclusions: Strong differences in protein expression and extent of phosphorylation were observed in the three tissues between the two feeding groups. Intensive feeding potentially led to a reduction of insulin sensitivity, indicated by a higher concentration of insulin and lower level of INSR. However, extent of phosphorylation of INSR was very low in both groups, which might be associated with fasting prior to slaughter.

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* *University of Hohenheim Animal Science, Stuttgart, Germany, sonja.baessler@campus.lmu.de*

88.

Using non-invasive methods to investigate telomere length in dairy cows and calves

Einsatz nicht-invasiver Methoden zur Bestimmung der Telomerlängen bei Milchkühen und Kälbern

*Häußler S., Bleikamp T., Gerlach K., Hölker M., Südekum K.-H., Sauerwein H. – Bonn

Telomeres are short repetitive DNA sequences covering the ends of chromosomes. Telomere length (TL) is considered as a biological marker for aging, shortening with every cell division. In dairy cows, TL has been shown to change in blood and different tissues throughout lactation (1). In order to test for less invasive methods, we collected nasal mucosa swabs and milk besides blood from dairy cows. After DNA isolation, the relative quantity of telomere (qT) products, reflecting TL, in nasal epithelial and in milk somatic cells was quantified and compared with qT from blood leukocytes. In addition, we aimed to investigate the changes in TL with age using a non-invasive method and thus analyzed the qT in nasal epithelial cells during the first 2 years of life in female Holsteins.

Methods: In Trial 1, multiparous German Holstein cows (n = 12; 2nd & 3rd lactation; average days in milk: 217 ± 11.8) were kept in a freestall barn and were fed a partial mixed ration (6.6 MJ NE_L/kg DM) offered for ad libitum intake and concentrate (7.7 MJ NE_L/kg DM) depending on the individual´s milk yield. Cows had a mean body weight of 657 ± 21.3 kg and a mean milk yield of 29.3 ± 1.23 kg/day. Blood samples were collected from the *V. caudalis*. Genomic DNA from whole heparinized blood was extracted using a commercial kit. From each cow, milk was sampled before blood collection. DNA from somatic cells in milk was isolated (First DNA all-tissue kit; gen-ial, Germany) after centrifugation (2000 x *g*; 20 min, room temperature). In Trial 2, calves (n = 16) were fed colostrum from day 1 - 3 post natum (p.n.), followed by transition milk until day 7 p.n.. From day 8 to 70 p.n. calves received milk replacer (150 g/L; 6 L/d followed by a linear reduction from wk 7 to 10 until 1 L/d) and had access to solid feed, i.e. grass hay and calf starter from day 14 p.n. onwards. After weaning around day 70 p.n., the calves were fed the same diet as used for lactating cows. Nasal mucosa epithelial cells were collected using swabs during calves` development in which each animal was sampled three times (age of animal at sampling 1: < 6 month; 2: sampling 1 + 6 month; 3: sampling 2 + 12 month). In both trials, samples from nasal mucosa were obtained by swabs (Performagene™ PG-100 swab collection kit, DNA Genotek, Canada), stabilized directly after sampling in Performagene™ solution and DNA was isolated according to the manufacturer's protocol. Isolated DNA was used to assess qT compared with the reference gene b-globin, using a multiplex qPCR (1). Statistical analysis was done using the general linear model for Trial 1. Associations between the different sample matrices were assessed by Pearson correlation. For evaluating changes of TL with increasing age, the regression between TL in nasal epithelial cells over age (minimum age: 2 d, maximum age: 2 years) was calculated ($P \leq 0.05$; SPSS 25).

Results: Comparing the different matrices in dairy cows, lowest qT were observed in blood leukocytes, whereas qT was 1.7-fold ($P < 0.001$) and 2.2-fold ($P = 0.038$) higher in nasal and milk epithelial cells, respectively. The relative qT in blood tended to be positively associated to the relative qT in nasal epithelial cells ($r = 0.532$; $P = 0.092$), however, qT in milk cells and blood leukocytes were not related. Comparing the qT in swab samples from female Holsteins throughout development, the negative slope in the linear regression ($y = -0.08 x + 283$) indicated decreasing TL during the first 2 years of life.

Conclusions: The lowest qT was observed in blood leukocytes, pointing to a higher turnover rate of bovine blood leukocytes when compared to nasal epithelial and milk somatic cells. The poor relationship between qT from nasal and milk epithelial cells with blood leukocytes lead to the conclusion that non-invasive samplings in the present study were not adequate to replace blood sampling. However, aging of female Holsteins was reflected in decreasing qT values from nasal epithelial cells.

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* *University of Bonn Institute of Animal Science, Bonn, Germany, susanne.haeussler@uni-bonn.de*

89.

Effects of essential fatty acid supplementation on the mRNA abundance of fatty acid binding receptors and the G protein-coupled lipid-binding receptor GPR18 in leukocytes of dairy cows fed a corn based ration

Effekte von essentiellen Fettsäuren auf die Expression der mRNA von Mitgliedern der Familie der fettsäurebindenden Rezeptoren sowie der GPR18 mRNA aus Leukozyten von Milchkühen, die mit einer auf Mais basierenden Diät gefüttert wurden

*Revskij D., Haubold S., Kröger-Koch C., Tuchscherer A., Tröscher A., Schuberth H.-J., Hammon H. M., Mielenz M. – Dummerstorf / Lampertheim / Hanover

A corn silage based diet lowers the available amount of n-3 fatty acids (FA) in dairy cows (1). Therefore, the pattern of plasma fatty acids as well as the fatty acid composition of the erythrocyte membrane is affected (1,2). Signaling by the family of free fatty acid receptors (FFAR1-4) and other lipid receptors can affect the immune response. The neutrophil release of granules is affected by long-chain and short-chain FA, which activate FFAR1-4. The lipid mediator resolvin D2, which derives from the n-3 FA docosahexaenoic acid, increases neutrophil phagocytosis and increase polymorphonuclear apoptosis after binding to G protein-coupled receptor GPR18. Therefore, resolvin D2 has pro-resolving function by activating GPR18. The dietary intake of FA and their metabolites may affect the pattern of potential ligands and the abundance of their receptors. Our objective was therefore to evaluate effects of a diet low in n-3 FA (trial 1) and of the same diet with additional FA supplementation (trial 2) on the mRNA abundance of FFAR1-4 and GPR18, expressed by bovine leukocytes.

Methods: In trial 1, cows were fed a grass silage (after calving) and a corn silage (CS) based total mixed ration (CS; from week 10 after calving on) for 24 weeks (n = 5). The buffy coat was collected from blood samples in weeks -1, 0, 1, 2, 8, 16, 24 relative to CS feeding. In trial 2, four cows were fed a CS diet. They were arranged in a 4 x 4 Latin square model and were supplemented with three successively rising FA dosages for two weeks, respectively, followed by a three-week wash out period on the CS diet. FA supplementation were either 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 buffy coat was isolated at the end of each treatment and wash out period from heparinized blood samples. The leukocyte mRNAs of FFAR1-4 and GPR18 (n = 5 in trial 1, n = 3 per treatment in trial 2) were quantified by real time PCR. Data were analyzed with the MIXED procedure of SAS using a repeated measurement analysis of variance model with weeks in milk as fixed effect in trial 1 and FA treatment and dosage as fixed effects in trial 2. Results are presented as LSMeans ± SEM.

Results: In trial 1, FFAR1 changed by weeks in milk as a trend ($P < 0.1$), with numerically lowest values at week 1 on the CS diet. GPR18 was affected ($P < 0.05$) by weeks in milk with numerically lowest values at week 24. In trial 2, FFAR2 was linked with the FA dosage ($P < 0.05$). The receptor GPR18 tended to change by time ($P < 0.1$). The abundance of GPR18 was lowest in the control group and highest in the EFA group ($P < 0.05$) and affected by the interaction of treatment x dosage ($P = 0.05$). The receptor FFAR4 was not detected by the used protocol.

Conclusions: Dietary FA influence the expression pattern of FFAR1, FFAR2 and GPR18. The ratio of n-6/n-3 FA and the resulting metabolites may affect the immune response in dairy cows because of their function as ligands for a panel of receptors expressed by leukocytes. Feeding a maize silage based may reduce signaling by GPR18 in dairy cows.

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* *Leibniz Institute for Farm Animal Biology (FBN) Institute of Nutritional Physiology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany, denis.revskij@med.uni-rostock.de*

90.

Studies on phytate disappearance and degradation products in growing pigs

Untersuchungen zum Abbau von Phytat im Verdauungstrakt von Schweinen

Rosenfelder-Kuon P., Klein N., Zegowitz B., Schollenberger M., Kühn I., *Rodehutscord M. – Stuttgart / Darmstadt

The majority of phosphorus (P) in plant feedstuffs is present as inositol hexakisphosphate ($InsP_6$), but $InsP_6$ concentrations differ between feedstuffs. Phytase is commonly used as a feed additive in pig diets to increase P digestibility through the release of P from $InsP_6$ until the end of the ileum. However, the increase in P digestibility due to phytase addition is smaller than the increase in precaecal $InsP_6$ disappearance. This indicates that inositol phosphates other than $InsP_6$ and with different degree of phosphorylation ($InsP_x$) remain undegraded until the end of the ileum. The objectives of this study were to identify $InsP_6$ degradation products at the terminal ileum and in the faeces of pigs when different levels of phytase were applied in maize-soybean meal (SBM)-based diets (Exp. 1), and when part of the SBM was replaced by rapeseed cake (RSC, Exp. 2).

Methods: Seven (Exp. 1) and eight ileally cannulated barrows (Exp. 2) with an initial body weight of 28 kg were used and assigned to four diets in each experiment in a completely randomised Double Latin Square design. The feed was provided twice a day in mash form and daily feed allowance was 4 % of body weight. Ileal digesta was collected during the last two days of a 12-day experimental period and faeces during the five preceding days. All diets were mixed without a mineral P supplement and contained about 0.6 % Ca and 0.5 % TiO_2 as inert marker. In Exp. 1, the four maize-SBM diets were supplemented with 0, 750, 1,500, or 3,000 FTU/kg of a modified *E.coli*-derived 6-phytase. Exp. 2 involved a 2×2-factorial arrangement of treatments. One factor was the main protein source (35 % SBM or a mix of 20 % RSC and 15 % SBM). The second factor was phytase supplementation (0 and 1,500 FTU/kg of the same phytase used in Exp. 1). Data underwent analysis of variance according to the respective experimental design using the Mixed procedure of SAS.

Results: In Exp. 1, precaecal $InsP_6$ disappearance significantly increased from 18 % without phytase to 83 % with phytase supplementation up to 1,500 FTU/kg. The corresponding precaecal P digestibility increased from 16 % to only 61 %. Phytase supplementation of 3,000 FTU/kg feed did not cause a further increase in either trait. In Exp. 2, no significant interaction between main protein source and phytase supplementation on $InsP_6$ disappearance and P digestibility was found. Precaecal $InsP_6$ disappearance increased from 31 % to 92 % with 1,500 FTU/kg feed, while P digestibility increased from 26 % to only 59 %. Precaecal P digestibility, but not $InsP_6$ disappearance, was lower when the diet contained RSC compared to SBM as the only protein source. In both experiments, the concentration of specific isomers of $InsP_2$, $InsP_3$, and $InsP_4$ in ileal digesta content significantly increased with phytase supplementation. The concentration of *myo*-inositol in ileal digesta also increased with phytase supplementation up to 1,500 FTU/kg. In both experiments, total tract $InsP_6$ disappearance was 97 % or higher and not significantly different between all treatments. However, this did not cause an increase in P digestibility compared to the corresponding precaecal P digestibility.

Conclusions: Phytase caused a remarkable increase in precaecal $InsP_6$ disappearance. While some $InsP_6$ was found completely dephosphorylated to *myo*-inositol at the terminal ileum, other parts were not at all or not completely dephosphorylated. Incomplete degradation of lower $InsP_x$ explains why P digestibility is not increasing to the same extent with phytase supplementation as $InsP_6$ disappearance increases. Data also demonstrate that intestinal phosphatases do not efficiently degrade lower $InsP_x$. Hindgut fermentation has the potential to degrade almost completely $InsP_6$ and $InsP_x$ that was not degraded until the terminal ileum, but this is not relevant for the supply of digestible P in pigs.

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* *Universität Hohenheim Institut für Nutztierwissenschaften, Stuttgart, Germany, markus.rodehutscord@uni-hohenheim.de*

91.

Diurnal patterns of feed intake and fecal pellet output in guinea pig: basic functional data for a frequently used species in gastrointestinal research

Tageszeitliches Muster des Futteraufnahme- und Kotabsatzverhaltens des Meerschweinchens: Basisdaten für eine häufig genutzte Spezies in der gastrointestinalen Forschung

*Elfers K., Armbrecht Y., Mazzuoli-Weber G. – Hanover

The guinea pig is a frequently used model in gastrointestinal research especially in studies on the enteric nervous system (ENS) (1). This includes both, basic research of ENS physiology as well as studies on pathological conditions of the ENS and connected functional symptoms. Hence, species-specific knowledge of basic parameters of gastrointestinal functions (such as feed intake and fecal pellet output and their possible correlation) is desirable. Unfortunately, only very few studies exist reporting on this topic in guinea pigs (2,3). The present study was conducted to gain basic data on feed intake and fecal pellet output in guinea pigs in order to build a fundament for further research in the field of gastrointestinal physiology including shift work model studies using this species.

Methods: guinea pigs, 3 to 5 months old, with a bodyweight of 500 to 800 g, were kept under standard conditions (55 % humidity, 22 °C, 12/12 h light/dark cycle with lights turning on at 7 a.m.) in dioecious groups of 2 to 4 animals, fed with a standard pelleted diet (g/kg TS: 168 crude protein, 31 crude fat, 130 crude fiber, 72 crude ash, 230 starch; 12.1 MJ ME/kg TS; ssniff Spezialdiäten) and with water *ad libitum*. On single animal basis feed intake (FI) and fecal pellet output (FPO) was determined in n = 14 guinea pigs of both sexes during the light period from 7 a.m. until 5 p.m, by hourly weighing the feed container and calculating the difference in weight and collecting fecal pellets, respectively. Animals were single housed 12 to 24 hours prior to the measurements to adapt to changed housing. Cumulative FPO/animal was calculated for the first 3 hours of fecal pellet collection. In a separate set of experiments FI and FPO was additionally determined during dark period in 4 single housed animals.

Results: during the light period guinea pigs showed a maximum FI of 0.61g/100 g body weight (BW)/hour during 7 and 8 a.m., while the lowest amounts were consumed between 11 and 12 a.m. (0.08g/100g BW). No differences between sexes were found. In the separate conducted trials with measurements during the dark period, animals showed continuous FI with a peak of FI around midnight. Comparison of FI during light and dark period revealed that all animals consumed approximately 60% of total FI during the light period. During light period FPO peaked between 8 and 9 p.m. with a mean of 16.5 pellets/animal and was lowest between 12 and 1 p.m. (2.6 pellets/animal). A significant correlation between FI and FPO could be computed ($p < 0.001$, $r = 0.37$). The four animals included in the 24 hours trial showed no defecation between 8 p.m. and 5 a.m. Cumulative FPO was 34.8 pellets/animal with no differences between sexes but great individual variations (from 0 to almost 90 pellets during the first 3 hours). The mean diameter of fecal pellets was found to be 5.01 ± 0.32 mm.

Conclusions: guinea pigs showed a clear, reproducible and sex-regardless distribution of FI with a "resting period" of 3 hours during the light period. As 60% of total FI was recorded during the light and 40% during the dark period, the guinea pig is more assignable to diurnal species. Recorded FPO peaked approximately 1 hour after the maximal FI indicating expected functional gastrocolic reflex. Cumulative FPO showed great individual variations indicating the importance to consider time point of collection and individual stress level of the single animal (e.g. influenced by changes in housing conditions) when using this parameter e.g. for evaluation of lower gastrointestinal motility. In further studies gastrointestinal transit time in guinea pigs under standard conditions should be evaluated in order to gain a holistic set of functional intestinal data of this species, which in the future might be used as a model for shift work in humans.

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* *University of Veterinary Medicine, Hannover, Foundation Institute for Physiology and Cell Biology, Bischofsholer Damm 15, 30173 Hanover, Germany, kristin.elfers@tiho-hannover.de*

92.

Investigations on the microbiome in the digesta of the small and large intestine of young pigs fed a rye based, fermented liquid diet, supplemented by coarsely ground cereals

Untersuchungen zum Mikrobiom des Dünn- und Dickdarminhalts junger Schweine bei Ergänzung des roggenbasierten, fermentierten Flüssigfutters durch grob vermahlenes Getreide

*Keller B., Bunte S., Grone R., Galvez E., Strowig T., Kamphues J. – Hanover / Braunschweig

Fermentation of liquid diets became more and more a common strategy for feeding fattening pigs (1). Especially this concept aims on improving the nutritive value of the diet by favouring protein and phosphorus digestibility (2). But there is also a weak point, i. e. the loss in particle size / physical structure during fermentation resulting in higher risks for gastric ulcers (1). Thus, there is a need to add coarsely ground ingredients to the fermented liquid diet prior to offering it to the pigs. Under these circumstances marked changes in the intestinal flora might be expected due to the high counts of active microorganisms in the fermented liquid diet but also due to the higher amounts of carbohydrates (starch, NSP, like fructans, arabinoxylans)) that reach the hindgut.

Methods: Two experimental studies with ten young individually housed pigs in each were performed (dietary treatment: 4 weeks). The first one aimed on the effects of the fermentation per se, the second one on consequences of adding 40 % roller mill treated cereals (rye, wheat, barley) to 60 % of fermented ingredients. In both trials identical diets were used in controls (non-fermented liquid diet) and the experimental group (60% fermented / 40 % fermented). The diet composition (%; rye 48.2; rapeseed, extracted meal 29.4; wheat 9.84; barley 9.80; mineral supplement 2.75) was oriented to reach common nutrient contents (200g crude protein, 6.7g calcium, 6.55g phosphorus) and was offered ad libitum (avoiding an empty trough). During the whole study feed intake (daily), gains (weekly) were performed. At the end all pigs were slaughtered to obtain samples of digesta from the small (ileum) and large intestine (colon). These digesta samples were stored at -80 C until chyme and excreta were homogenized. DNA-extraction was done on a liquid handling automate; based on the DNeasy Blood&Tissue Kit. Amplification of the 16S rRNA gene was done on hypervariable region V 4. The amplicons were sequenced on the Illumina-Miseq platform. DNA reads processing and statistical analysis was performed using QIIME (version 1.8.0) and MicrobiomeAnalyst.

Results: In the final diet at offering to the pigs counts of lactic acid bacteria differed markedly (non fermented diet: $10^{4.91}$ / fermented diet $10^{9.31}$ cfu/g), correspondingly also the lactic acid concentrations differed (0.15g/kg DM vs. 53.7 g/kg DM) Due to the addition of roller mill treated cereals markedly higher amounts of coarse particles (~ 25 % > 2mm) were found in the wet sieve analysis. The microbiome of pigs fed the liquid diet was characterized by a "normal" diversity including higher shares of gramnegative bacteria, whereas in pigs fed the fermented diet the microbiome in the small intestine was completely different, i. e. dominated by the flora of the fermented diet. Of special interest were the market differences regarding the microbiome of the colonal digesta: Due to the addition of coarse cereals (mainly rye) there was a marked shift, especially the high shares of *Lactobacillaceae* is worth to be mentioned.

Conclusions: Without any doubt the high counts of active lactic acid producing bacteria and the high concentration of dietary lactic acid determine predominantly the flora within the small intestine, it means the fermented liquid diet has to be considered as "probiotics enriched" diet. On the other hand supplementation of the fermented liquid diet with coarsely ground rye acts like a high effective prebiotic, selectively favoring *Lactobacillaceae* as it is intended by multiple feed additives. Fermentation of a liquid diet and the use of coarsely ground rye might be considered as highly effective dietetic measures suitable to favor "gut health" in general.

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* *University of Veterinary Medicine Hannover, Foundation Institute for Animal Nutrition, Bischofsholer Damm 15, 30173 Hanover, Germany, birgit.keller@tiho-hannover.de*

93.

Treatment of IPEC J2 cells with Berberine to analyze its effects on the epithelial barrier

Behandlung von IPEC J2 Zellen mit Berberin, um Effekte auf die epitheliale Barriere zu untersuchen

*Cornelius V., Amasheh S. – Berlin

Berberine is a plant alkaloid, which occurs naturally in different Berberis species. It is used in traditional eastern medicine as a treatment against diarrhea. Recently, a barrier-strengthening effect of berberine has been reported in human intestinal Caco-2 and HT29 B6 cell monolayers [1, 2]. Aim of our study was the analysis of the effect of berberine on the epithelial barrier of the porcine intestine. Therefore, we chose the improved porcine intestinal cell model of IPEC J2 cells [3]; by using medium supplemented with porcine serum instead of fetal calf serum, the epithelial properties of the cell line turned out to be more comparable with those of the porcine small intestinal epithelium.

Methods: 10^5 IPEC J2 cells, cultivated in DMEM/Ham´s F12 supplemented with 10 % porcine serum and 1 % penicillin/streptomycin, were seeded on Millicell® PCF- culture plate inserts. After 14 days of growth, the trans-epithelial electrical resistance (TEER) was measured with a chopstick electrode and an epithelial volt-ohm meter (EVOM). When the TEER was stable, a 24 h-incubation with 0, 50, 100 or 200 µM of berberine chloride, solved in DMSO, was started. The TEER was measured after 2, 4, 6, 10 and 24 h and subsequently, the cells were prepared for immunohistological stainings or western blot analysis. In addition, the viability of the cells was analyzed with an ApoToxGlo-assay (Promega). Statistical testing was performed with one-way ANOVA and Dunnett´s test for multiple comparisons. Values below $p = 0.05$ were considered to be statistically significant.

Results: After 6 h of incubation with berberine, the TEER of the IPEC J2 monolayers started to descend in a dose-dependent manner ($p < 0.05$ for 100 µM and 200 µM); after 24 h, the TEER value was significantly lower for all tested concentrations ($p < 0.01$) compared to controls. Western blot analysis of tight junction proteins revealed no significant reduction of zonula occludens protein 1 (ZO-1) and claudin-1. Using immunohistological stainings and confocal laser scanning microscopy, the signal for ZO-1 could be located next to apicolateral membrane compartments. In contrast, claudin-1 signals were located in the paracellular space under control conditions but not after treatment with 200 µM berberine. To further investigate the mechanism, an ApoToxGlo assay was performed after 6 h of treatment with different berberine concentrations. Whereas no cell toxicity could be detected, the viability decreased and the caspase-3 and -7 activity increased dose-dependently.

Conclusions: In contrast to the barrier-strengthening effect of berberine in human intestinal cell model [1, 2], the effect in IPEC J2 cells seems to be barrier-weakening. The results of the ApoToxGlo assay indicate the induction of apoptosis by berberine. If the barrier-weakening effect induces the apoptosis, or the cellular change due to the apoptosis results in a weaker barrier, needs to be elucidated. Based on the tight-junction associated ZO-1 signal and the intracellular localization of the claudin-1-signal in immunohistological stainings after berberine treatment, the involvement of claudin-1 appears likely.

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* *Freie Universität Berlin Institute of Veterinary Physiology, Oertzenweg 19b, 14163 Berlin, Germany, valeria.cornelius@fu-berlin.de*

94.

IPEC-J2 cells as a model to investigate the effects of TNFα on porcine intestinal epithelial barrier function

IPEC-J2 Zellen als Modell für die Untersuchung der Effekte von TNFα auf die porcine intestinale epitheliale Barrierefunktion

*Drößler L., Cornelius V., Amasheh S. – Berlin

The porcine jejunal cell line IPEC-J2 is a non-transformed intestinal cell line which grows as a monolayer separating an apical and a basolateral compartment (1). Thus they provide a suitable model for the study of intestinal epithelial transport and barrier functions (1). Recently, effects of barrier weakening compounds on intestinal epithelial barrier properties have been analyzed employing this model (2). The current project focuses on effects of the cytokine Tumor Necrosis Factor alpha (TNFα), which is a main mediator in intestinal inflammatory responses and downregulates barrier properties in different cell lines (3). The aim of this study was the analysis of time- and concentration-dependent TNFα effects on barrier function in IPEC-J2 cell monolayers.

Methods: Cells were grown until confluency on semipermeable cell culture inserts with a pore size of 0.45 µm.For analyzing the effect of TNFα, different concentrations of the cytokine were added (500, 1000 and 5000 U/ml) and effects on transepithelial resistance, representing the epithelial barrier function, were monitored for 96 h using a chopstick electrode and an epithelial volt-ohm meter. At different time points, cells were harvested for immunoblotting detecting tight junction proteins claudin-1, -3 and zonula occludens protein 1 (ZO-1). In parallel, confocal laser-scanning immunofluorescence microscopy was performed to examine the localization of the proteins. Statistical analysis was performed using one way ANOVA and Dunnett's test for correction of multiple testing. P-values of < 0.05 were considered to be statistically significant.

Results: 1000 U/ml TNFα induced a decrease of transepithelial resistance over 96 h. With a higher concentration (5000 U/ml) this effect could be observed already after 48 h. This functional outcome was reflected by effects on total expression and apicolateral localization of tight junction proteins claudin-1, -3 and ZO-1.

Conclusions: Our study reveals that the non-transformed porcine epithelial cell line IPEC-J2 provides a useful model for analysis of inflammatory effects on epithelial barrier function. The outcome on molecular level is in accordance with functional changes and with general barrier-perturbing properties of TNFα.

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* *Freie Universität Berlin Institute of Veterinary Physiology, Oertzenweg 19 b, 14163 Berlin, Germany, linda.droessler@fu-berlin.de*

95.

Effects of increasing rye levels in pelleted diets for fattening pigs on the composition of gastric digesta, including so-called 'doughballs'

Auswirkungen steigender Roggenanteile in pelletiertem Mischfutter für junge Mastschweine auf die Zusammensetzung des Magenchymus, einschließlich sogenannter „doughballs"

*Wilke V., Grone R., Kamphues J. – Hanover / Bergen

The unique carbohydrate fraction of rye, especially its high levels of arabinoxylans and fructans have an impact on physical properties of the digesta, e.g. on the viscosity, pH and DM- content in the anterior gastro intestinal tract of pigs [1]. Moreover it is known that hydro-thermal treatment of feeds affects 'clumping behavior' of stomachs' digesta [2]. The experimental studies, mentioned below, aimed on a characterization of the stomach contents including the formation/composition of 'doughballs'.

Methods: 2 x 40 pigs (trial 1: age: 46.8 ± 5.28 days; bodyweight (bw): 16.1 ± 4.13 kg / trial 2: age: 48.7 ± 1.38 days; bw: 16.5 ± 2.65 kg) were housed individually in four groups of 10 pigs each. Trial 1: each group was fed a diet consisting of wheat and/or rye, barley, soy, potato protein and a mineral supplement. The sum of wheat and rye was 69 % in all diets, whereby the compound feed of each group was characterized by different wheat : rye- ratios (100/66.6/33.3/0). Trial 2: All pigs were fed a diet containing 60 % of rye, soybean meal (SBM) and/or rapeseed meal (RSM), moreover barley, lignocellulose and a mineral supplement. Different shares (%) of both protein rich ingredients (SES/RSM: 18.1/0; 13.6/6.70; 8.10/16.1; 0/28.0) characterized these diets. Over the entire experimental period, feed intake (daily) and gains (weekly) were determined individually. After 4 weeks of dietary treatment, the pigs were dissected and the digesta of the stomach was collected for characterization. The prevalence of 'doughballs' was documented and stomach health was determined. Furthermore the liquid content and the 'doughballs' were analyzed separately for pH and DM-content (2nd trial). Statistics were done by SAS® Enterprise Guide® (Anova; $p < 0.05$).

Results: In the first trial 'doughballs' were found mainly in the groups with higher shares of rye in the diets (1st group: 1 of 10; 2nd group: 2 of 10; 3rd group: 5 of 10; 4th group: 9 of 10). 'Doughballs' were found nearly equally in all groups of the second trial, where a rye based diet (60 % rye) was fed (8-10 of 10). The pH and DM-content showed significant differences between the 'doughballs' and the liquid stomach contents (trial 2). The pH within the 'doughballs' varied at 6.00 ± 0.278 while in the liquid stomach content lower values (4.58 ± 0.349) were found. The DM-content of the 'doughballs' was almost doubled compared to the liquid phase ('doughballs': 481 ± 42.0 g/kg; liquid stomach content: 222 ± 31.4 g/kg). No significant differences regarding stomach health were found between the groups in both trials (in general: moderate – high hyperkeratosis).

Conclusions: 'Doughballs' were found mainly in the groups with higher dietary rye levels (trial 1), resulting from 'clumping behavior' of the digesta. Different shares of SBM and RSM did not affect the occurrence of 'doughballs' (trial 2). The difference in the composition of stomach contents might be of interest regarding potential consequences for the protein digestion that starts in the stomach with the formation of pepsin at low pH values only. As is known the precaecal digestibility of protein from rye is lower than in wheat and further cereals. Maybe the higher DM contents in the digesta lead to a delayed stomach transit time (longer filling) which could be an advantage, e.g. in feeding of pregnant sows. However the daily feed intake was not negatively affected in both trials. Specific effects of the 'doughballs' on gastric health (e.g. alterations/ulcers) were not observed. Finally it has to be underlined that high dietary rye levels did not result in 'doughballs' formation, when non-pelleted diets were used, offered in a liquid form [3].

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* *University of Veterinary Medicine Hannover, Foundation Institute for Animal Nutrition, Hanover, Germany, volker.wilke@tiho-hannover.de*

96.

Effects of substituting wheat for rye in compound feeds on nutrient digestibility (praecaecal/ total tract) in Göttingen minipigs

Effekte eines Ersatzes von Weizen durch Roggen im Mischfutter auf die Verdaulichkeit (praecaecal/ gesamt) bei Göttinger Miniaturschweinen

*Hartung C. B., Grone R., Wilke V., Kamphues J. – Hanover / Bergen

The particular chemical composition defining rye (e.g. non-starch polysaccharides (NSPs) such as arabinoxylans and fructans) is of growing interest in pig nutrition. These distinct NSPs contribute to a forced formation of butyrate in the hindgut [1]. Butyrate is said to have diverse beneficial effects regarding *Salmonella* prevalence, boar taint and behaviour [2]. Previous trials with growing pigs have repeatedly shown a significantly lower ileal digestibility of crude protein and amino acids for rye in comparison to wheat [3]. The aim of this study was to compare the apparent ileal (AID) and total tract digestibility (ATTD) of nutrients in two diets containing 69% wheat or 69% rye in pigs. Rye (15.1 MJ ME/kg DM) and wheat (15.5 MJ ME/kg DM) provide similar energy densities with lower values regarding crude protein, crude fat and starch for rye.

Methods: Three adult Göttingen minipigs (31.3±1.93 kg, 6.3±1.5 years) were fed two different conventional diets containing either 69% wheat (F1) or 69% rye (F2) as well as barley (10%), soy (11.5%), potato protein (5.2%) and a supplement with minerals and amino acids (2.8%) to reach almost isoenergetic and isonitrogenous levels**.** The individually housed pigs were fitted with an ileo-caecal fistula and received both diet consecutively. The pelleted diets were ground to pass through a 0.5 mm sieve and fed to the pigs in 200 g servings (+ 1 L of water and 0.625 g of Cr_2O_3) twice a day. After adaptation (4d), digesta collection (3d) started, during which the pigs were fed at 7:00h and the fistulae were opened. For the following 12h the whole ileal digesta was collected and at 19:00h the fistulae were closed. In a further study, faeces were collected individually over a five-day period after one week of adaptation from the same pigs to determine the ATTD. The dry matter content of digesta and faeces was measured, then crude nutrients were analysed in the lyophilised material. AID was calculated with the marker method and ATTD with the collection method. Statistical analyses were performed using the SAS® software (ANOVA); data were normally distributed.

Results: Significant differences ($p < 0.05$) were found for AID of organic matter (OM) and N-free extractives (NfE) with lower values for the rye diet (4.7% lower for NfE; 3.7% lower for OM) but not for the other nutrients. The lower AID of NfE and OM in F2 led to an increased inflow of these nutrients into the large intestine (1.25-fold and 1.17-fold, respectively) where the substrate was provided for fermentation. For the ATTD respective significant differences could not be found (only 0.7 and 1.1 percent points lower for NfE and OM). However significantly lower values were found for digestibility of crude fat in F2 (10.7% lower than F1). As those differences could not be found for AID, this fat may stem from microbial synthesis and fixation.

Conclusions: The AID of F1 and F2 did not show significant differences for most of the nutrients, including protein, which is contrary to recent findings [3]. Nevertheless it has to be emphasized that a complete feed was used where only 40.7% (rye) or 52.5% (wheat) of the crude protein originated from the respective cereal, whereas the remainder derived either from soy/potato protein or added crystalline amino acids. It can be concluded that substituting wheat for rye in high proportions does not result in reduced digestibility rates for pigs when the diets are adequately supplemented (with amino acids). The significantly lower AID of OM and NfE led to an increased inflow into the large intestine. The high fermentation is able to compensate those differences- with the above mentioned positive effects so that no higher amounts of faeces are produced ("feed for the large intestine") despite lower digestibility rates in the small intestine. The higher postileal digestion in rye fed pigs might be even more pronounced at higher feed intake/when diets are offered ad libitum.

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* *University of Veterinary Medicine, Hannover, Foundation Institute for Animal Nutrition, Bischofsholer Damm 15, 30173 Hanover, Germany, clara.berenike.hartung@tiho-hannover.de*

97.

Activity of Disaccharide Degrading Enzymes in the Digestive Tract of Desert Locusts (*Schistocerca gregaria*)

Aktivität Disaccharid-spaltender Enzyme im Verdauungstrakt von Wüstenheuschrecken (Schistocerca gregaria)

*Paulicks B. R., Dinglreiter M., Windisch W. – Freising

When insects should be used as food or feed in the future, a large-scale production with adequate feeding will be necessary. However, knowledge about the digestive capacity of insects is still limited. It is of particular interest whether insects may digest non-starch-polysaccharides (NSP), since degradation of these types of carbohydrates is limited to microbial digestion in the forestomaches of ruminants and - to a smaller degree – in the caecum and colon of all livestock and of humans. As locusts generally consume fibrous material (e.g. grass), one could assume a general potential to extract dietary energy from NSPs. Indeed, recent studies (1) suggest a cellulolytic digestive potential of locusts, but a more detailed characterization is still lacking. Therefore, the present study aimed into assessing the digestive potential of locusts for the disaccharides cellubiose, the most simple representative of cellulose, saccharose, lactose, and maltose, a representative of starch.

Methods: Nine adult desert locusts (*Schistocerca gregaria*) were immobilized for 5 minutes in a freezer (-22 °C) and killed by decapitation. The entire digestive tract was removed, homogenized, diluted with distilled water, and centrifuged. The supernatant, containing the water-soluble constituents including digestive enzymes, was mixed either with four aqueous disaccharide solutions (cellubiose, maltose, saccharose, lactose) or with pure water (control, baseline). Release of glucose was monitored photometrically every 4 minutes using a glucose test kit over a time span of 24 minutes. Means per substrate and time were calculated using Windows Excel program. The slope of the regressions of each substrat by time was evaluates for statistical significant difference using GLM procedure of SAS.

Results: Addition of cellubiose resulted in a clear release of glucose over the entire time of observation ($p<0.0001$). Linear release of glucose was observed also with maltose ($p<0.0001$), saccharose ($p<0.0001$), and lactose ($p<0.001$). Corresponding speed of glucose release accounted for 656%, 333%, and 45% as compared to cellubiose. Speed of glucose release was statistically different among all disaccharides tested ($p<0.001$).

Conclusions: Although locusts as generally regarded as grass consumers expecting a large capacity to digest roughage, i.e. feedstuff rich in cellulose, the present results show, that their carbohydrate digestion capacity is mainly adapted to amylolytic feed carbohydrates (e.g. starch) and saccharose. Nevertheless, also some cellulolytic enzyme activity was proved. The question, whether locusts are also able to digest other soluble non-starch-polysaccharides such as e.g. inulin, pectins etc., needs further investigations.

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* *Technical University Munich Animal Nutrition, Liesel-Beckmann-Str. 2, 85354 Freising, Germany, paulicks@wzw.tum.de*

98.

Effects of biochar as a minor component of compound feed in young fattening pigs on nutrient digestibility and performance parameters

Auswirkungen von Pflanzenkohle als Mischfutterkomponente auf die Nährstoffverdaulichkeit und Leistungsparameter bei jungen Mastschweinen

*Schubert D. C., Witte F., Terjung N., Hankel J., Visscher C. – Hanover / Quakenbrück

In order to prevent boar taint, biochar was tested an intestinal binding agent for skatole and indole- Therefore, it was to proof if biochar could take negative effects either on performance parameters or nutrient digestibility. Biochar is defined as a heterogeneous substance produced by biomass pyrolysis, which is a process that requires temperatures between 350 and 1000 °C and low oxygen levels [1]. Furthermore, biochar is known for its well pronounced, unspecific binding capacity and several fields of applications have been described in literature [2].

Methods: This study was conducted in 18 male, intact, mixed breed pigs with an initial body weight (BW) of 6.88 ± 1.17 kg and 21 days of age. The animals were housed individually and had *ad libitum* access to water and feed. The study itself was split into two parts. Within the first part the animals were divided into three groups and assigned to one of the three experimental feedings (Control feed = CON, feed containing biochar 1 = BC 1, feed containing biochar 2 = BC 2). BC 1 and BC 2 contained 2 % of the according biochar. The chemical composition of the feed was 5.3 %, 5.1 % respectively 5.2 % crude ash, 19.4 %, 17.9 % respectively 17.7 % crude protein (XP), 4.3 %, 4.1 % respectively 4.1 % ether ectract (EE) and 4.7 %, 4.6 % respectively 4.5 % crude fibre (XF). After ten days, every group received CON for the consecutive four days. By using a cross-over design, each group was assigned to another feed for day 15-24, followed by another four days of CON for all groups. This procedure was repeated one last time so that after three feeding cycles every group received all three feeds. Within each cycle a digestibility study was performed. The first five days of feeding served as an adaption period whereas during the following five days faeces were collected completely and individually. BW was measured at days 0, 5, 10, 14, 20, 25, 28, 34, 39 and 42 and feed intake was determined daily. By start of the second part of the study, the animals were assigned to three new groups that contained two animals of each previous group. Every group received one of the experimental feed for the following four weeks. During this time, BW and feed intake were determined weekly. At the end of the study, all animals underwent slaughter.

Results: There were no significant differences found in feed intake, daily weight gain and feed conversion ratio between the three groups. However, there were significant differences found for apparent total tract digestibility (ATTD) of dry matter (DM) and macronutrients as well as of phosphorous and zinc ($p < 0.05$; ANOVA; SAS® Enterprise Guide®). ATTD of DM (CON: 82.9 ± 2.8 %^{A}, BC 1: 85.7 ± 2.1 %^{B}, BC 2: 87.1 ± 2.4 %^{B}), organic matter (83.8 ± 2.8 %^{A}, 86.5 ± 1.9 %^{B}, 87.9 ± 2.3 %^{B}), EE (70.8 ± 3.6 %^{A}, 75.8 ± 3.1 %^{B}, 77.7 ± 3.1 %^{B}), XF (30.8 ± 13.4 %^{A}, 38.4 ± 8.2 %^{B}, 40.4 ± 12.2 %^{B}) and N-free extractive (89.1 ± 2.1 %^{A}, 91.5 ± 1.3 %^{B}, 92.6 ± 1.5 %^{B}) was significantly higher for both BC 1 and BC 2 in comparison to CON, while digestibility of XP was merely significantly higher for BC 2 compared to CON (81.0 ± 4.1 %^{A}, 82.4 ± 3.6 %^{AB}, 84.2 ± 3.4 %^{B}). For ATTD of calcium, iron and copper no statistically significant differences were found. These results are against our expectations, especially the higher digestibility of DM, since biochar is an inert, therefore non-digestible, substance.

Conclusions: It was to proof if biochar as a feed additive in a relative high concentration of 2 % has negative effects in growing pigs. In this study, no such negative consequences could be found, but on the contrary, the digestibility of macronutrients could be improved.

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* *University of Veterinary Medicine Hannover, Foundation Institute for Animal Nutriton, Bischofsholer Damm 15, 30173 Hanover, Germany, dana.carina.schubert@tiho-hannover.de*

99.

In vitro gas production from batch cultures with stomach and hindgut digesta of horses adapted to a prebiotic dose of fructooligosaccharides and inulin

In vitro Gasproduktion von Batch-Kulturen mit Digesta aus dem Magen und Dickdarm von Pferden, die an eine präbiotische Dosierung von Fruktooligosacchariden und Inulin adaptiert waren

*Bachmann M., Glatter M., Bochnia M., Greef J.-M., Breves G., Zeyner A. – Halle (Saale) / Braunschweig / Hanover

Fructooligosaccharides (FOS) and inulin are used as prebiotics to modulate hindgut fermentation. However, if these prebiotics remain unprotected, they do not fully pass the stomach and small intestine (1). The objective was to study if this is reflected by in vitro fermentation using inocula from stomach and hindgut segments of horses adapted to FOS and inulin.

Methods: Twelve horses with 534 ± 64.5 kg body weight (BW) and 14 ± 7.5 years of age were fed meadow hay (1.5 kg/100 kg BW/d) and crushed oats (1.2 g starch/kg BW/d) for 20 d. They were euthanized on the 21st d after the morning meal. Six of the 12 horses additionally received 0.15 g FOS + inulin/kg BW/d via Jerusalem artichoke meal (JAM). The other 6 horses got corncob meal without grains (CMG) as placebo. Digesta from stomach, cecum, ventral colon ascendens (VCA), and colon transversum (CT) was sampled from separated gut sections, mixed with CAESITEC buffer-nutrient solution under CO_2-flush, filtered, and incubated (39 °C, 50 rpm) using the ANKOM^{RF} Gas Production System (3 × 6 replicates per segment and group). To each fermenter (340 mL volume), 1.09 g cellulose and 0.19 g corn starch were added (i.e., 0.7 % cellulose and 0.1 % starch in 153 mL inoculum). Gas was pressure-dependently released and gas production was monitored every 5 min. After 48 h, fermentation was stopped and the fluids were sampled. The pH and oxidation-reduction potential (ORP) were measured before and after the fermentation. Starch, simple sugars (i.e., glucose + fructose + sucrose) and fructans were analyzed in digesta using the amyloglucosidase method and HPLC-RID, respectively. Statistical analysis was performed with SAS 9.4 using fixed time, treatment, segment, time × treatment × segment effects, and a random animal effect in the model at $P < 0.05$ significance level.

Results: In stomach digesta, concentrations of starch, simple sugars, and fructans were 155 vs. 154 g/kg dry matter (DM), 34 vs. 33 g/kg DM, and 35 vs. 53 g/kg DM in CMG vs. JAM, respectively. In CMG vs. JAM batch-cultures, total gas production was 380 vs. 325 mL in stomach ($P < 0.01$), 285 vs. 297 mL in cecum ($P > 0.05$), 239 vs. 272 mL in VCA ($P = 0.05$), and 209 vs. 200 mL in CT fermenters ($P > 0.05$). In stomach fermenters, more gas was produced with JAM vs. CMG between 4 and 24 h of incubation ($P < 0.05$), whereas in the hindgut fermenters, the progress of gas production did not differ ($P > 0.05$). Pre-incubation pH was 6.0 in stomach, but between 6.7 and 6.9 in hindgut fermenters ($P < 0.001$). Post-incubation pH was 4.3 (CMG) and 4.0 (JAM) in stomach, but between 5.7 and 6.0 in the hindgut fermenters ($P < 0.01$). Neither pre-incubation nor post-incubation pH differed between CMG and JAM. In the hindgut fermenters, ORP ranged from -239 to -311 mV. It did not differ between CMG and JAM, between pre-incubation and post-incubation stages, or among the hindgut segments. In the stomach fermenters, however, ORP differed between CMG and JAM, both at pre-incubation and post-incubation stages ($P < 0.05$). In the JAM group, ORP increased from -245 before to -99 mV after 48 h of fermentation ($P < 0.001$).

Conclusions: The elevated gas production with stomach inocula from prebiotic fed horses during an early fermentation stage might reflect the greater fructan content (1), as well as a shift in the microbial community (2). In vitro hindgut fermentation confirmed previous suggestion of little impact of FOS and inulin in an apparently prebiotic dose when given unprotected (2).

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* *Martin Luther University Halle-Wittenberg Institute of Agricultural and Nutritional Sciences, Theodor-Lieser-Straße 11, 06120 Halle (Saale), Germany, martin.bachmann@landw.uni-halle.de*

100.

Effects of neonatal glutamine supplementation on the jejunal abundance of mRNA encoding for antioxidative defence enzymes, tight junction and glutaminase proteins in low birth weight piglets

Effekte einer neonatalen Glutaminsupplementierung auf die jejunale mRNA-Expression von Enzymen der antioxidativen Abwehr, Tight-Junction Proteinen und Glutaminase bei Ferkeln mit niedrigem Geburtsgewicht

*Schregel J., Sciascia Q. L., Li Z., Metges C. C. – Dummerstorf

The primary organ for nutrient absorption is the intestine, and its development is delayed in low birth weight (L) piglets (1). Glutamine (Gln) is known to be a fuel for rapidly dividing intestinal cells (2) and has also been shown to beneficially affect piglet intestinal health (3), however most studies have focused on the effects during weaning. Therefore, we assessed acute and persistent effects of neonatal glutamine supplementation on the abundance of mRNA encoding proteins for anti-oxidative defense, tight junction molecules and glutamine metabolism in the jejunum of L piglets.

Methods: At birth (day of life (d) 0), male L (0.8-1.2 kg, n=48) and normal-birth weight (N; 1.4-1.8 kg, n=48) littermates born to gilts were selected. The next day, litter size was standardized to 12 piglets, and experimental piglets were randomly assigned to oral supplementation groups (d 1 to 11) with Gln (1 g/kg body weight (BW)/d) or alanine (Ala; 1.22 g/kg BW/d; isonitrogenous to Gln): Gln-L, Gln-N, Ala-L or Ala-N. Piglet BW, crown-rump length (CRL), abdominal circumference (ABC) and IUGR (intrauterine growth retardation) score were measured at birth. Subgroups (n=48) were euthanized at d 12 and 26 and jejunual tissue samples were taken, frozen and RNA was isolated. The relative mRNA abundance of *GPX1, GPX2, GSRa, CGL, CuSoD* (antioxidative defense)*, Claudin-4*, *Zo-1, Zo-2, Occludin* (tight junctions)*,* glutaminase (Gln metabolism) were analysed by rt-qPCR. Data was analyzed using the MIXED procedure of SAS. Least square means were separated using the Tukey test ($P<0.05$).

Results: At birth N piglets were heavier, longer (CRL) and wider (ABC) than L (Ala-N vs Ala-L; 1.6 vs 1.1 ± 0.08 kg; 24.8 vs 22.6 ± 0.56 cm; 24.4 vs 22.1 ± 0.52 cm, Gln-N vs Gln-L; 1.5 vs 1.1 ± 0.08 kg; 24.8 vs 22.4 ± 0.56 cm; 24.2 vs 22.0 ± 0.52 cm, $P < 0.05$). At d 12 no difference in mRNA abundance was observed among all groups. However, at d 26 Gln–L showed lower mRNA abundance of the antioxidant markers *GPX1* ($P<0.1$), *GPX2* ($P<0.1$) and *GSR1a* ($P<0.05$) compared to Ala-L. The mRNA abundance of antioxidant *GPX-2* ($P<0.1$), *GSRa1* ($P<0.05$), *CGL* ($P<0.05$), and tight junction *Claudin-4* ($P<0.05$), *ZO-2* ($P<0.001$), *Occludin* ($P<0.1$) and *CuSoD* ($P<0.1$) markers were lower in Ala-N than in Ala-L. There were no differences in the gene abundance between Gln-L and Gln-N. The abundance of glutaminaseand *ZO-1* mRNA did not differ between groups.

Conclusions: Glutamine supplemented L piglets at d 26 may be less challenged by oxidative stress than Ala-L. However, tight junction markers were lower in Ala-N compared to Ala-L contradicting other studies, which requires further investigation.

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* *Leibniz Institute for Farm Animal Biology (FBN) Institute of Nutritional Physiology, Wilhelm Stahl Allee 2, 18196 Dummerstorf, Germany, schregel@fbn-dummerstorf.de*

101.

Effects of systemic inflammation on mitochondrial DNA copy number in leukocytes of dairy cows

Effekte systemischer Entzündung auf die mitochondriale DNA-Kopienzahl in Leukozyten von Milchkühen

Seibt K. D., Alaedin M., Ghaffari M. H., Frahm J., Dänicke S., Huber K., *Sauerwein H. – Bonn / Braunschweig / Stuttgart

The copy number of the mitochondrial genome, the mitochondrial DNA (mtDNA), reflects the abundance of mitochondria within a cell and is affected by environmental, physiological, and energetic conditions. The ratio between mitochondrial and nuclear genomes is a suitable measure for the mtDNA content[1]. In dairy cows the comprehensive changes in energy balance (EB) during lactation have been demonstrated to be related to mtDNA[2]. The early postpartum period is characterized by a systemic inflammation, but negative energy balance at that state versus inflammation cannot be experimentally dissected. To investigate the interrelationship between mtDNA and inflammation, we thus tested the effects of a controlled inflammatory stimulus on leukocyte mtDNA at mid lactation when EB has reached positive values, and compared against peak lactation when EB is still negative but the parturition-related inflammation has ended. In addition, we aimed to test the effects of supplementing L-carnitine which facilitates the beta-oxidation of long-chain fatty acids in the mitochondria by transporting the substrate into the mitochondria.

Methods: Blood samples from in 50 pluriparous Holstein cows (mean number of lactations: 2.56) were collected at days +42, and +100 after to calving. The cows were receiving either a L-carnitine supplement (25 g/day rumen-protected L-carnitine, Carneon 20 Rumin-Pro, Kaesler Nutrition GmbH, Cuxhaven, Germany) from d -42 until the end of the observation period (n = 26) or no supplement (n = 24). A further blood sample at day -42 before calving was limited to 22 cows (11 per group). After taking the day +100 sample, all animals were subjected to an intravenous challenge with lipopolysaccharide (LPS; *E. coli* O111:B4, Sigma-Aldrich, 0.5 µg/kg BW) and a further sample 48 h later was collected. DNA was isolated from heparinized full blood and mtDNA was assessed by multiplex qPCR, i.e., amplifying mitochondrial 12s rRNA together with β-globin, a housekeeping gene that is used as the nuclear control gene with a known copy number of 2 per cell. Strict criteria of validation were applied as described earlier[3]. Statistical analyses using SPSS were performed using linear mixed models with time, treatment, parity and the respective interactions thereof as fixed effects.

Results: The mtDNA values from day -42, +42 and +100 were not different, but the values obtained 48 h after the LPS challenge were lower than on all preceding times ($P < 0.001$) irrespective of carnitine supplementation. Taking also the factor parity into account, time, parity and the interaction between time and parity were significant, but neither treatment nor the respective interactions were significant. Cows with more than 2 lactations had consistently less mtDNA than those in their second lactation with the exception of d 100 ($P < 0.001$).

Conclusions: Our results indicate that mtDNAwas decreased by the LPS challenge at mid lactation but was neither altered by carnitine supplementation nor the negative EB around peak lactation. It remains to be elucidated as to whether negative EB during early lactation, when the peripartal inflammation is still ongoing, might also affect mtDNA.

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* *University of Bonn Institute of Animal Science, Katzenburgweg 7, 53115 Bonn, Germany, sauerwein@uni-bonn.de*

102.

Immunometabolic challenge: Glucose levels and neutrophil phagocytic activity in ewes supplemented with magnesium during the transition period

Immunstoffwechselstress: Glukosespiegel und neutrophile phagozytische Aktivität bei Mutterschafen, die während der Übergangszeit mit Magnesium gefüttert wurden

*Ahmed M., Breves G., Wilkens M., Schuberth H.-J., Ganter M. – Hanover

The transition period (TP) is characterized by many metabolic and endocrine changes. In addition to an enhanced risk for metabolic disorders such as pregnancy toxemia, this might contribute to immunodysregulation or – suppression by affecting myeloid cell functions such as neutrophil phagocytosis. Magnesium (Mg) requirements of the ewes also increase significantly during the TP resulting in a greater risk to develop subclinical/clinical hypomagnesemia. Interestingly, Mg supplementation was used successfully to improve the glucose metabolism in humans, As it is essential for more than 300 metabolic reactions associated with functions of energy and glucose metabolism, insulin function, ion transport, cell signaling and migration. Since glucose oxidation is one of the main sources for ATP synthesis in neutrophils, this study aimed at analyzing whether dietary Mg supplementation modulates glucose metabolism and neutrophil phagocytic activity during the TP in ewes.

Methods: Nineteen healthy pregnant German Blackhead ewes (entering the 2nd and 3rd lactation), were divided into 2 groups, control group (n=9) and Mg group (n=10). In accordance with NRC, (2007) recommendations, the control group received 4.25 g Mg daily ante partum (a.p.) and 6.24 g Mg daily post partum (p.p.). The ration of the Mg group was additionally supplemented with Mg oxide resulting in a daily Mg intake of 5.37 g and 8.62 g, respectively. Blood samples were collected 5 times: on day (d) 30 a.p., d 14 a.p., d 1 p.p., d 14 p.p. and d 30 p.p. Serum concentrations of Mg, calcium, and glucose were determined using an autoanalyzer/spectrophotometer. Whole blood neutrophil phagocytic activity (uptake of FITC- conjugated and heat killed *Staphylococcus aureusin vitro*, 50:1 (Bacteria: cell) was evaluated by flow cytometry. Unpaired t test and two way RM ANOVA (GraphPad Prism 8) were used for comparison between the different time points and groups.

Results: Mg serum levels were not affected by the treatment. In both groups, Ca serum levels were lowest on d 1 p.p, ($p < 0.05$). In comparison to the control group, ewes supplemented with Mg showed lower glucose concentrations ($p = 0.07$). Flow cytometric analysis revealed that neutrophil phagocytic activity was lowest on d 30 a.p. and on d 1 p.p. ($p < 0.001$-0.05, respectively) in the control group. Interestingly, on d 1 p.p the neutrophil phagocytic activity (percentage of positive phagocytic neutrophils and the mean phagocytic capacity per cell) was higher ($p < 0.05$) in the Mg group compared to the control group.

Conclusions: We were able to show that dietary Mg supplementation might modulate glucose metabolism and alter some immune functions such as neutrophil phagocytic activity around parturition. As activated immune cells rely on glycolysis, this may thus influence the ewes ability to cope with some pathogens.

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* *Unviversity of Vetreinary Medicine Hannover Institute of Physiology and Cell biology, Bischofsholer damm 85, 30173 Hanover, Germany, mona.hassan.ahmed@tiho-hannover.de*

103.

Short-term responses of fatty acid profiles and phenol concentrations in cows' milk to different tanniferous forages

Kurzzeitreaktion auf verschiedene tanninhaltige Futterpflanzen in Fettsäurenprofil und Phenolgehalt der Milch von Kühen

*Birkinshaw A., Schwarm A., Marquardt S., Kreuzer M., Terranova M. – Zurich / Ås / Nairobi

In many parts of the world, cow's milk and other dairy products are considered staple foods. Cows play an important role in subsistence farming in developing countries and rural communities where dairy products considerably contribute to human nutrition. In industrialized societies, diets high in dairy products feature significantly, too. Fatty acids like α-linolenic acid (C18:3n-3) and rumenic acid (C18:2c9t11), the most important isomer of the conjugated linoleic acids, are considered particularly beneficial to human health. Others, like linoleic acid (C18:2n-6), are more abundant in western diets and can compromise health when consumed in such high proportions. Although biohydrogenation occurs in the rumen, the fatty acid profile of cow's milk can be manipulated by the cow's diet (1). Independently from modifying the dietary fat type, this also seems possible by dietary phenols which may counteract ruminal biohydrogenation of polyunsaturated fatty acids (PUFA). However, it is not known how quickly the fatty acid profile changes, if this change is dose related and if phenols can be recovered in the milk.

Methods: Late-lactating dairy cows were used in two different experiments. In the first experiment, based on a Latin Square design, six cows were fed six different diets each. A basal diet (grass silage, maize silage, hay and concentrate) was maintained and pellets, in a ratio of 0.4:0.6 to the basal diet, were added. Pellets consisted of lucerne exclusively or lucerne combined with one of six phenolic plants before pelleting. These plants were wood avens (*Geum urbanum*) and rosebay willow (*Epilobium angustifolium*) herbs, and leaves of blackcurrant (*Ribes nigrum*), green grape vine (*Vitisvinifera*), silver birch (*Betula pendula*) and hazel (*Corylusavellana*). It was intended that the phenolic plant pellets had a total extractable phenol content of 60 g/kg total diet dry matter (DM). Diets were offered for 3 days each. In the second (22-day) experiment, 20 cows were fed the same basal diet (and basal diet to pellet ratio) with one of 20 lucerne-based pellet types with increasing proportions (0 to 410 g/kg DM) of hazel leaves. Milk was sampled on the first and last day of feeding (both experiments). Milk was analysed for fatty acids and total phenol content, and diets for proximate composition and total extractable phenols. Data were evaluated in SAS version 9.4 using the Mixed procedure with Tukey-Kramer adjustment for multiple comparisons among means (Experiment 1) and multiple regression analysis (Experiment 2).

Results: Three days of feeding phenolic plants was sufficient to cause dietary related changes to milk fatty acid profiles in Experiment 1. Most individual milk fatty acids were not significantly affected by the phenolic plants, only C18:3*n*-3 and C18:2*n*-6 were higher in proportion of total fatty acids when cows were fed the first four phenolic plants, listed above, but decreased when hazel and silver birch were fed. This may indicate that the first four plants helped preventing part of the PUFA from being biohydrogenated. C18:2c9t11 proportions decreased in all six diets; the same trend was found for C18:1*t*11, its precursor in the rumen. The phenolic plants led to a numerical increase in milk phenol content. Increasing hazel proportions in the diet caused a linear trend ($P = 0.07$) towards an increase in phenol concentration in the milk (Experiment 2). However, there was no significant response in the proportions of key fatty acids to dietary hazel proportions. Most plants did not affect palatability (except silver birch and blackcurrant), however, at high doses digestibility decreased (2).

Conclusions: These results indicate that dietary manipulation can be successfully applied to favourably modify cows' milk fatty acid profiles and phenol concentration, in a short period of time. However, this does not seem to be dose dependent as the tanniferous hazel leaves did not change these milk properties at higher dosages.

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* *ETH Institute of Agricultural Sciences, Universitätstrasse 2, 8092 Zurich, Switzerland, amy.birkinshaw@usys.ethz.ch*

104.

Effects of a systemic inflammatory challenge on the concentrations of the acute phase protein haptoglobin in serum and milk of dairy cows receiving a L-carnitine supplement

Auswirkungen eines systemischen Entzündungs-Challenge auf die Konzentrationen des Akute-Phase-Proteins Haptoglobin in Serum und in Milch von Kühen unter L-Carnitin-Supplementierung

Alaedin M., Blees T., Ghaffari M. H., Huber K., Dänicke S., Frahm J., *Sauerwein H. – Bonn / Braunschweig / Stuttgart

Haptoglobin (Hp) is one of the major acute phase proteins in cattle. The concentrations in milk were elevated during mastitis and were also shown to increase upon intramammary challenge with lipopolysaccharide (LPS) earlier than in blood[1]; indeed, local expression in the mammary gland parenchyma was demonstrated[1] both at the mRNA and the protein level. Beside, LPS-induced alterations of the blood-milk barrier may support a transfer of Hp from blood to milk. If systemic inflammation would affect the Hp concentrations in milk, the use of Hp measurements in milk as supporting indicator for mastitis would be compromised. With this background we aimed to assess the concentrations of Hp in serum and in milk of dairy cows in response to an intravenous challenge by LPS.

Methods: Multiparous Holstein cows were allocated to 2 feeding groups (each n= 26), i.e. one group with and the other one without supplementation of rumen-protected L-carnitine (25 g/day rumen-protected L-carnitine, Carneon 20 Rumin-Pro, Kaesler Nutrition GmbH, Cuxhaven, Germany) from d 49 ante partum until the end of the observation period (d 120 of lactation). All cows were intravenously injected with LPS (*E. coli* O111:B4, Sigma-Aldrich, 0.5 µg/kg BW) on day 110 of lactation. Blood samples were taken at -24 h and +12, +24, +72, and +192 h relative to the LPS challenge, milk was sampled at -12 h, 0, 24, 60, 72, 180, and 192 h. Hp was quantified by ELISA[1]. For milk, values of -12 and 0 h were combined for obtaining baseline values. Cows with elevated baseline serum Hp concentrations (> 1 mg/mL, n = 2 in the carnitine group; n = 3 in the control group) indicative for inflammation before the LPS challenge were excluded from the statistical analyses. The MIXED procedure of SAS with cow as random effect was used with group, time, and group x time as fixed effects.

Results: The serum Hp concentration increased with time ($P<0.01$) after the LPS challenge reaching peak values 8.6-fold greater than before LPS at 72 h irrespective of feeding group. In milk, Hp concentrations also changed with time ($P<0.01$) without differences between groups reaching peak values at 180 h (2.2-fold of basal). Even though an increase in milk Hp was observed upon systemic LPS challenge, the fold change at peak was much less than observed earlier with intra-mammary LPS application already after 12 h (170-fold) with concomitant increases in serum Hp to 11-fold of basal[1].

Conclusions: The increased HP concentrations in milk in systemic inflammation in the present study are in support of an impaired blood milk barrier rather than stimulated local secretion. Concerning the fold-increase observed herein in milk, the use of measuring milk Hp concentrations for investigating mastitis or as a supportive diagnostic tool for mastitis -besides somatic cell counts -, is not compromised in presence of systemic inflammation.

Acknowledgement: This contribution is submitted on behalf of the DFG-funded MitoCow project.

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* *University of Bonn Institute for Animal Science, Katzenburgweg 7, 53115 Bonn, Germany, sauerwein@uni-bonn.de*

105.

Effects of supplementation of a green tea extract (GTE) on milk yield and hepatic expression of genes involved in inflammation and endoplasmic stress response in dairy cows

Einfluss eines Grüntee-Extraktes auf die Milchleistung und die Expression von Genen der Entzündung und der Antwort auf Stress des endoplasmatischen Retikulums in der Leber bei Milchkühen

Geßner D. K., Brock C., Koch C., *Eder K. – Giessen / Münchweiler an der Alsenz

The transition period represents the most critical period in the productive life of high yielding dairy cows. Over the first weeks of lactation all cows develop a negative energy balance which leads to metabolic stress with undesirable effects on liver metabolism. Therefore, the production of cytokines in the liver is enhanced, which in turn leads to the occurrence of inflammation and endoplasmic reticulum (ER) stress which adversely influences the health status of cows. Previous studies have shown that supplementation of plants or plant extracts rich in polyphenols are able to attenuate the inflammatory process and ER stress in the liver of dairy cows during early lactation. Green tea is particularly rich in polyphenols, and the present study investigated the hypothesis that supplementation of a green tea extract (GTE) has the potential to suppress the inflammatory process and ER stress in the liver of dairy cows during early lactation.

Methods: Thirty multiparous dairy cows (German Holstein) were divided into a control group (n = 15) and a group receiving GTE (n = 15; 10 g/d from wk 1 a.p. to calving, 20 g/d from calving to wk 1 p.p.). The average number of lactation in these groups was 2.80 and 3.33, respectively. All cows received a total mixed ration. GTE (with a polyphenol content of 351 mg gallic acid equivalents per g of product) was mixed into 500 g of concentrate which was administered to the cows by hand after milking. The control group received the identical amount of concentrate without supplement. Milk samples were taken weekly from wk 1 to wk 7 p.p. Blood samples (from *vena caudalis mediana)* and liver biopsies (from the right liver lope) were taken in wks 1, 4 and 7 p.p. after the morning milking before feeding. Relative mRNA concentrations of hepatic genes were determined by qPCR. Data were statistically evaluated by the Linear Mixed-Effects Model of R (version 3.1.1). The model included treatment, week, lactation number and the treatment x week interaction as fixed factors and cow as random factor.

Results: Dry matter intake, milk yield and protein concentration in the milk did not differ between the two groups of cows in wks 2 to 7 of lactation. However, the GTE group showed a lower fat concentration in the milk (3.68 vs. 4.29%, SE = 0.18%, $P < 0.05$), a lower daily amount of fat (1.58 vs. 1.96 kg/d, SE = 0.10 kg/d, $P < 0.05$), a tendency towards a less negative energy balance (-35.0 vs. -45.1 MJ NEL/d, SE = 7.0 MJ NEL/d, $P = 0.06$) in wks 2 to 7 of lactation and a reduced plasma NEFA concentration (0.43 vs. 0.52 mM, SE = 0.06 mM, $P < 0.05$) compared to the control group. Relative mRNA concentrations of genes involved in inflammation, including genes of acute phase response and genes of the Nrf2 pathway of the antioxidant system in the liver remained unchanged between the two groups of cows. However, relative mRNA concentrations of some genes of the unfolded protein response (UPR) which are triggered by ER stress (*DNAJC3, HSPA5, CASP3*) were reduced in wk 1 in the cows supplemented with GTE ($P < 0.05$). Moreover, cows supplemented with GTE showed a tendency towards a reduced concentration of triglycerides (TAG) in the liver in wks 1 to 7 ($P = 0.07$). As relative mRNA concentrations of genes of lipid metabolism in the liver remained largely unchanged between the two groups, it is suggested that the trend towards a reduced TAG concentration in the liver was due to the improved energy balance in the cows supplemented with GTE.

Conclusions: The study shows that supplementation of GTE overall has no favorable effects on milk performance. The reduction of milk fat concentration suggests that GTE could influence milk fat synthesis in the liver by a mechanism which cannot be explained by the data of this study. The finding that some genes of the UPR were down-regulated in GTE supplemented cows supports previous findings showing that dietary polyphenols are able to attenuate ER stress in dairy cows during early lactation.

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* *Justus Liebig University Giessen Institute of Animal Nutrition and Nutrition Physiology, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany, klaus.eder@ernaehrung.uni-giessen.de*

106.

Relationship between the occurrence of stress of the endoplasmic reticulum in the liver and milk performance, energy balance, inflammation, the antioxidant system and vitamin D and calcium homoestasis in dairy cows during early lactation

Beziehung zwischen dem Auftreten von Stress des endoplasmatischen Retikulums in der Leber und der Milchleistung, der Energiebilanz, der Entzündung, dem antioxidativen System sowie der Vitamin D- und Calciumhomöostase bei Milchkühen während der Frühlaktation

Geßner D. K., Hof L. M., Koch C., *Eder K. – Giessen / Münchweiler an der Alsenz

A previous study has shown that stress of the endoplasmic reticulum (ER stress) is induced in the liver of dairy cows during early lactation. It has been assumed that the unfolded protein response (UPR) which is triggered by ER stress contributes to pathophysiologic conditions commonly observed in the liver of periparturient cows. However, the reasons for the induction of ER stress in the liver of dairy cows are yet unknown. The present study aimed to investigate potential physiologic reasons of the induction of ER stress in the liver of dairy cows during early lactation.

Methods: 50 Holstein cows [19 primi- and 31 multiparous (lactation number 2-5)] were included in the animal experiment which was conducted at the at the Educational and Research Centre for Animal Husbandry Hofgut Neumühle (Münchweiler an der Alsenz, Germany). The animals were fed a total mixed ration which met the nutritional requirements according to GfE. Milk yield and feed intake were recorded. Blood and liver samples taken 1 wk post partum (day 6 - day 10) were considered for this study. The relative mRNA concentration of heat-shock protein 5 (*HSPA5*) in the liver, a robust marker of the occurrence of ER stress, was considered to assign the cows into three groups with low, intermediate or high level of ER stress. Relative mRNA concentrations of genes were determined by qPCR, concentrations of 25-hydroxy vitamin D3 (25-OH-D3), 1,25-dihydroxy vitamin D3 (1,25-OH-D3), PTH and FGF23 were determined by ELISA kits. The three groups were analysed for different traits and biochemical pathways; means were compared by one-way ANOVA followed by Fisher's multiple comparison test.

Results: The relative mRNA concentrations of HSPA5 in the three groups were 1.00 ± 0.32 (n = 11, low ER stress, mean ± SE), 2.02 ± 0.20 (n = 28, intermediate ER stress), 5.32 ± 0.32 (n = 11, high ER stress). Cows with high ER stress had higher relative mRNA concentrations of several other genes of the UPR (*ATF4*, *DNAJC3*, *EDEM1*, *HERPUD1*, *PDIA4*, *XBP1*) in the liver than cows with low or intermediate ER stress ($P < 0.05$), confirming that these cows had the highest level of ER stress. The three groups with different ER stress levels did not differ in milk yield in wk 2 and 100-day milk yield energy balance as well as plasma NEFA and BHBA concentrations in wk 1. Cows with high ER stress showed higher relative mRNA concentrations of haptoglobin, ceruloplasmin and C-reactive protein, three acute phase proteins, in the liver than cows with low or intermediate ER stress ($P < 0.05$), whereas relative mRNA concentrations of pro-inflammatory genes (*TNF*, *IL1B*, *IL8*) did not differ. Concentrations of antioxidants (α-tocopherol, β-carotene), the total antioxidant capacity and concentrations of thiobarbituric acid-reactive substances and protein carbonyls in plasma, relative mRNA concentrations of antioxidant genes (*CAT*, *SOD*, *GPX1*) in the liver as well as several parameters of the vitamin D and calcium homeostasis (plasma concentrations of calcium, phosphate, 25-OH-D3, 1,25-OH-D3, PTH and FGF23) did not differ between the three groups. However, hepatic concentrations of triglycerides and cholesterol in the liver were higher in the group of cows with high ER stress than in the group with low ER stress ($P < 0.05$).

Conclusions: The data of this study show that the induction of ER stress in the liver of dairy cows is unrelated to milk yield and energy balance of dairy cows, and there was also no relationship with the antioxidant system and the vitamin D and calcium homeostasis. The finding that cows with a high ER stress level showed higher relative mRNA concentrations of acute phase proteins indicates that an acute phase reaction could stimulate the induction of ER stress in dairy cows. Moreover, it was found that the ER stress is associated with a higher triglyceride and cholesterol concentration in the liver which indicates that ER stress could be involved in the development of a fatty liver.

* *Justus Liebig University Giessen Institute of Animal Nutrition and Nutrition Physiology, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany, klaus.eder@ernaehrung.uni-giessen.de*

107.

Timecourse of the serum concentrations of acute phase proteins and metabolites in water buffalos during the transition period

Verlauf der Serumkonzentrationen von Akut-Phase-Proteinen und Metaboliten wärend der Transitionsphase bei Wasserbüffeln

*Sauerwein H., Blees T., Zamarian V., Catozzi C., Müller U., Sadri H., Dänicke S., Frahm J., Ceciliani F. – Bonn / Milano / Tabriz / Braunschweig

The transition from pregnancy to lactation implies comprehensive metabolic and endocrine changes including a proinflammatory reaction and oxidative stress around calving in dairy cows. Our objective was a longitudinal characterization of the concentrations of acute phase proteins (APP), i.e., haptoglobin (Hp), serum amyloid A (SAA) and acidic glycoprotein (AGP) in another large dairy animal, i.e. in water buffalos (*Bubalus bubalis*), in context with indicators for metabolic stress (nonesterified fatty acids (NEFA) and ß-hydroxybutyrate (BHB) and adiponectin) during that phase.

Methods: For the valid determination of the target proteins, bovine specific ELISA in-house developed methods were validated and in case of Hp and adiponectin modified (Hp: purification of buffalo Hp for calibrating a serum standard; Hp and adiponectin: consistent use of buffalo serum for coating and standard). For SAA and AGP, commercially available ELISAs (Life Diagnostics Ltd., Stoke on Trend, UK: Cow AGP-11 and Cow SAA-11 were used). Blood samples were collected weekly from 11 pluriparous water buffalo cows (lactation number 4.6 ± 1.6; daily milk yield 9.0 ± 1.9 kg) from 6 weeks (wk) ante partum (ap) until 8 wk post partum (pp). Linear mixed models with time (wk) as repeated effect considering the nested periods ap and pp and cow as random effect were used to evaluate the time courses of the different variables using IBM SPSS25. The level of significance was set at $P \leq 0.05$.

Results: For Hp and SAA peak values were observed in the first week pp; Hp concentrations were greater ap as compared to the weeks following the peak; SAA values before and after the peak were not different. For AGP an increase was observed from the 1st to the 2nd wk pp with values remaining elevated above ap until the end of the observation period. From the metabolic indicators,adiponectin concentrations were not affected by time, whereas time was significant for NEFA and BHB with greater values observed ap than pp. The time course of NEFA and BHB point to greater metabolic load in late pregnancy as compared with the first weeks of lactation - contrary to the common situation in Murrah buffalos and dairy cows. Both BHB and NEFA values remained below the thresholds applied for dairy cows to define subclinical or clinical ketosis thus indicating that the buffaloes studied herein were not under particular metabolic stress.

Conclusions: The increase in concentration of the acute phase proteins around calving is in support of the notion that inflammation is a physiological epiphenomenon of the onset of lactation in water buffalos.

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* *University of Bonn Institute for Animal Science, Physiology & Hygiene, Katzenburgweg 7, 53115 Bonn, Germany, sauerwein@uni-bonn.de*

108.

Influence of different methionine sources on inflammatory markers in different small intestinal regions of pigs

Einfluss verschiedener Methioninsupplemente auf Entzündungsmarker im Dünndarm des Schweins

*Schermuly I. I., Romanet S., Klünemann M., Whelan R., Aschenbach J. R. – Berlin / Hanau

Methionine (Met) as an essential and one of the first limiting amino acids is an important feed additive for poultry and swine. Met is involved in several important biochemical pathways involving transamination, transmethylation and transsulfuration reactions. Metabolites from the Met pathways like glutathione and taurine play essential roles in the regulation of anti-oxidant reactions and help maintain gut health. While an important fraction of the dietary Met is directly metabolized in the intestine, its hydroxy analog HMTBA (2-hydroxy-4-methylthiobutanoic acid) appears to be mainly processed in the liver and kidney. The present study investigated whether different Met sources influence gene expression linked with inflammatory responses in the small intestinal tissue.

Methods: Experiments were performed on 27 Danbred x Pietrain male castrated piglets with an initial age of 10 weeks. Piglets were randomly separated into three feeding groups. Each group received the same basal diet that was formulated to meet the requirements of the German Society of Nutrition Physiology (GfE; 18.0% CP, 10.3 MJ/kg NE) but restricted in sulfur amino acid content (0.26% Met, 0.30% Cys). To meet Met+Cys requirements, one of the following Met supplements was added: 0.21% L-Met, 0.21% DL-Met or 0.31% DL-HMTBA. Diets were fed over a pre-feeding period of at least 10 days. The gene expression of samples from the epithelium of duodenum (DUO), proximal jejunum (PJ), middle jejunum (MJ) and ileum (ILE) were analyzed by qPCR for the following genes: IL-1β, IL-8, IL-18, NLRP3, CASP1 and TGF-β. For normalization, the following unregulated housekeeping genes were used: GAPDH, β-actin and YWHAZ. Data were compared by Two-way ANOVA with post-hoc Student-Newman-Keuls' test as appropriate.

Results: Irrespective of diet, the relative gene expression differed between intestinal segments for only IL-18 and TGF-β. IL-18 transcripts were higher in PJ compared to MJ and ILE ($P < 0.05$), while TGF-β expression was higher ($P < 0.05$) in ILE in comparison with other small intestinal segments. When comparing gene expression among feeding groups, IL-18 transcript levels were higher in all intestinal epithelia from the DL-HMTBA and DL-Met feeding groups compared to samples from animals supplemented with L-Met ($P < 0.05$). DL-Met supplementation of the diet induced a higher expression of TGF-β compared to the L-Met and DL-HMTBA-containing diets ($P < 0.05$). Feed supplementation had no significant effect on the expression of the other investigated genes.

Conclusions: Expression of immune related genes varies only moderately among the different intestinal segments. Feeding different Met sources induced some moderate changes in the relative expression of inflammation-related genes in the intestinal epithelium. The changes included a lower expression of pro-inflammatory IL-18 in the L-Met-supplemented group and a higher expression of anti-inflammatory TGF-β in DL-Met-supplemented pigs, both of which may indicate a reduced inflammatory status of the small intestine when feeding those supplements.

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* *Freie Universität Berlin Institute of Veterinary Physiology, Oertzenweg 19b, 14163 Berlin, Germany, isabel.schermuly@fu-berlin.de*

109.

Influence of polyphenols (hydroxytyrosol and carnosic acid) supplementation on reproductive performance of sows

Einfluss von Polyphenolen (Hydroxytyrosol und Carnosinsäure) auf die Fortpflanzungsfähigkeit von Sauen

*Cañete González J., González-Añover P., López-Andreo M. J., Ramis Vidal G. – Tarragona / Zaragoza / Murcia

Swine production has markedly increased its reproductive performance over the past 50 years. One of the most important limitations to continue increasing the size of the litter, or to fully express the genetic potential of the breeders, is the prenatal mortality of embryos. Among other causes the antioxidant/oxidant balance has a key role in this. Among other causes the antioxidant/oxidative balance has a key role (1,2,3), alternative sources of antioxidants are polyphenols and, particularly, the current study aimed to analyze the usefulness of hydroxytyrosol and carnosic acid during gestation supplementation in the reproductive performance of sows in commercial herds.

Methods: A total of 97 female breeding pigs from the 1st to the 7th litter were allocated under the same conditions simultaneously into two treatment groups during the whole gestation period to compare the effects of supplementation in the feed with hydroxytyrosol and carnosic acid (MiaPhenol; 150 mg/kg feed DM; total amount of phenols in product 35 mg/g DM; group MPH) and control group. Both treatments received the same basal diet formulated to meet the requirements during pregnancy, while having *ad libitum* access to water. The daily amount of feed supplemented during the entire gestation phase was 2.5 kg per animal and day. Data were collected individually for total number of born piglets, live-born piglets, stillborn piglets and mummified piglets at the farrowing moment (<12 h post farrowing). Data were analyzed using a statistical package SPSS v. 15.0. Comparisons of means were made using a Student's t-test, with prior verification of similarity of variances using the Levene test. Significant differences of less than 0.05 were always considered significant.

Results: Throughout the treatment, the group MPH showed higher number of total born piglets per litter (18.32 vs. 16.39; P=0.02), as well as, number of live-born piglets per litter (16.66 vs. 14.78; P=0.04) compared to control group. Number of stillborn and mummified piglets per litter did not significant differs among groups (1.41 vs. 1.23; P=0.59; 0.23 vs. 0.37; P=0.25 respectively).

Conclusions: The present trial indicates that the supplementation with hydroxytyrosol and carnosic acid during gestation period improve significantly both number of total born piglets and live-born piglets. Moreover, the addition of polyphenols do not affect stillborn and mummified number of piglets per litter. Further studies are needed to clarify mode of action and efficacy of the product.

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* *Miavit Nutrición Animal S.L., Tarragona, Spain, juan.canete@miavit.es*

110.

MitoCow# - Changes of ruminal and duodenal microbiota and metabolites during two physiological challenges in dairy cows

MitoCow# - Änderungen der Mikrobiota des Pansens und Duodenums während zwei physiologischen Stresssituationen in Milchkühen

*Tröscher-Mußotter J., Deusch S., Camarinha-Silva A., Frahm J., Dänicke S., Huber K., Seifert J. – Stuttgart / Braunschweig

Environmental and physiological stressors are challenging the dairy cow's health and thereby performance, in particular during transition period and infections. Feeding regimens are discussed more frequently considering the complex microbial ecosystem hosted in these animals. Preceding studies demonstrated highly intricate communication pathways between the host and its symbiotic inhabitants. Yet, the microbial community dynamics of the ruminant's intestine during health and stress are hardly investigated so far. The aim of this study was to observe the microbial composition and metabolites in rumen and duodenum fluid challenged by transition to lactation and by intravenous (iv) lipopolysaccharide (LPS)-induced inflammation. Each animal was sampled intensively before, during and after both events. Obtained data sheds light into the complexity of microbial and metabolic shifts in the rumen and duodenum of dairy cows during challenging phases.

Methods: Eight rumen and duodenum fistulated Holstein cows were sampled for rumen and duodenal fluid at day 42, 14 *antepartum* (*ap*); 14, 100, 118 and 126 *postpartum* (*pp*) and 12, 24, 72 hours after calving as well as an i.v. LPS injection (day 111 *pp*, using 0.5 µg/kg body weight). The cows were divided into a control and L-carnitine supplemented group (n=4 each, 25g/cow/day). The ration was composed of 80% roughage and 20% concentrate from day 42 *ap* until the day of calving. Thereafter, the concentrate level was gradually increased up to day 14 *pp* to 50%, which was maintained until the end of the trial. Bacterial DNA was extracted from rumen and duodenal fluid samples. The 16S rDNA gene was sequenced by Illumina amplicon sequencing. Sequences were clustered into operational taxonomic units (OTUs). Nuclear magnetic resonance (NMR) was applied for metabolomic analyses. Multivariate statistical analysis were used to analyse different data sets.

Results: Data analysis resulted in 10 bacterial phyla, 42 families and a total of 4,278 OTUs. Microbiota of the rumen and duodenal fluid changed considerably during transition period and throughout the complete trial period ($p=0.0001$; $R=0.6$). Carnitine supplementation did not reveal an impact, neither on the microbial composition nor on metabolite patterns. The α-diversity showed a tremendous herd homogeneity until calving. The gradual increase in concentrate feed proportion after calving resulted in a steady decrease of diversity in both matrices. This was accompanied by a higher herd standard deviation within, indicating individuals coped differently during transition period, whereas this effect was not as prominent during the LPS challenge. Sixty metabolites (e.g. fatty and amino acids, urea), indicated clear shifts along the trial period in both groups. Rumen and duodenal fluid samples were not significantly different at the microbiota ($p=0.0002$, $R=0.09$) but rather on the metabolome level ($p=0.0001$, $R=0.9$).

Conclusions: The combination of Illumina sequencing and NMR, revealed that microbial communities and metabolites fluctuated equally across the herd but also individually during transition period and an i.v. LPS-induced inflammation. Carnitine supplementation did not have an effect, neither on microbial nor on metabolome-level. This project represents the first long-term study enlightening microbial and metabolic interactions in dairy cows during multiple challenges.

Name of joint project

* *University of Hohenheim Institute of Animal Science, 70599 Stuttgart, Germany, johanna.troescher@uni-hohenheim.de*

111.

MitoCow[#] - Effect of a L-carnitine supplementation on blood parameters indicative for lipid metabolism in *intravenous* lipopolysaccharide challenged cows

MitoCow[#] - Einfluss von supplementiertem L-Carnitin auf den Fettstoffwechsel betreffende Blutparameter bei Milchkühen nach einer intravenösen Lipopolysaccharidinjektion

*Meyer J., Daniels S. U., Frahm J., Kersten S., Kluess J., Most E., Eder K., Meyer U., Rehage J., Dänicke S. – Braunschweig / Hanover / Giessen

High yielding dairy cows are metabolically challenged by the high energy demand for milk production, which might affect their responsiveness to immunogenic stimuli and therefore to infectious. This experiment was designed to examine the hypothesis, that a dietary L-carnitine supplementation modulates the endogenous response to lipopolysaccharides (LPS) due to an enhanced mitochondrial energy metabolism, which might result in an improved energy availability.

Methods: For the experiment 51 pluriparous German Holstein cows were assigned to a control (CON) and carnitine group (CAR) considering similar numbers of lactation, body condition score (BCS), body weight and fat-corrected milk yield of previous lactation. Experimental feeding started six weeks *ante partum* (*ap*) with a ration consisting of 80% roughage (70% maize and 30% grass silage) and 20% concentrate on a dry matter basis until day one *post partum* (*pp*). Thereafter, the proportion of concentrate increased from 30% up to 50% (final proportion) within the first two weeks *pp*. The L-carnitine (25g/day rumen-protected L-carnitine, Carneon 20 Rumin-Pro, Kaesler Nutrition GmbH, Cuxhaven, Germany) was supplied via concentrate feed from automatic feeding stations while the remaining ration was fed as a partial mixed ration. To compensate the fat component in the carnitine product, the supplementary concentrate of CON contained an equivalent quantity and quality of fat (BergaFatF-100 HP 98, Berg+ Schmidt GmbH & Co. KG, Hamburg, Germany). Water was offered for *ad libitum* intake during the whole experiment. Cows in both groups were challenged by an injection of 0.5 µg/kg body weight of LPS (E.coli, Serotyp O111:B4, Sigma Aldrich, St. Louis, Missouri, USA) in one *Vena jugularis externa* in week 16 *pp*. Blood samples were collected on day 1 before LPS injection, after LPS injection 0.5, 1, 2, 3, 4, 6, 9, 12, 24, 48, 72 hour/s (h) and 7 and 14 days after LPS injection, where the cows were also clinically examined. Furthermore, the BCS was determined once a week, milk samples were analyzed twice a week and body weight and milk yield were recorded twice a day. Concentrations of free carnitine (CA) in EDTA plasma were measured by tandem mass spectrometry according to Hirche et. al (2009). Non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB) and triacylglycerides (TG) were detected photometrically (Eurolyser CCA 180 (Eurolyser Diagnositca GmbH, Salzburg, Austria) in serum. Statistical analyses were carried out by MIXED-Model procedure of SAS 6.1 with time (as repeated measurement), group and the interaction of group and time as fixed factors. Least square mean comparisons were carried out by means of Tukey-Kramer test.

Results: LPS induced a systemic inflammatory response as indicated by time-dependent changes in all measured parameters and further indicators (see Daniels, this issue). L-carnitine supplementation significantly increased blood CA levels (7-fold, $p < 0.001$) and reduced the NEFA-levels ($p=0.044$) while the concentrations of BHB and the other parameters remained uninfluenced ($p > 0.05$). BHB reached its maximum level, which was 67% higher than the BHB concentration at day 1 before LPS injection, 48 h post LPS injection. The highest TG level occurred already 0.5 h post LPS injection and was 36% higher than the initial level. NEFA in CON was 14% higher than in CAR.

Conclusions: The decrease in NEFA in CAR and similar BHB levels between both groups might hint at an increased efficiency of energy utilization. Further evaluations will focus on the energy utilization in more detail to substantiate this hypothesis.

[#]Name of joint project

* *Friedrich-Loeffler-Institut Institut für Tierernährung, Bundesallee/ 37, 38116 Braunschweig, Germany, jennifer.meyer@fli.de*

112.

MitoCow# - Effect of a L-carnitine supplementation on haematological profiles of dairy cows challenged by bacterial lipopolysaccharides (LPS)

MitoCow# - Einfluss von einer L-Carnitin Zulage auf die Hämatologie von Milchkühen nach einer Lipopolysaccharid (LPS)-Injektion

*Daniels S. U., Meyer J., Frahm J., Kersten S., Kluess J., Meyer U., Huber K., Dänicke S. – Braunschweig / Stuttgart

Inflammation, as induced e.g. by LPS, is associated with an increased energy requirement. At cellular level, energy can be supplied by β-oxidation of fatty acids in which L-carnitine can be a rate limiting factor. Furthermore, L-carnitine is an antioxidant and could prevent for dysfunctions and membrane lesions. Therefore, we hypothesized that the leukogram of L-carnitine supplemented dairy cows are differently affected by the influence of the metabolically challenge during an inflammation by LPS.

Methods: For a feeding experiment 54 pluriparous German Holstein cows were assigned to a control (CON, n = 27) or a L-carnitine group (CAR, n = 27). From six weeks *ante partum*, all cows were fed a ration consisting of 80% roughage (70% maize and 30% grass silage) and 20% concentrate on dry matter basis, with or without L-carnitine (25 g/day of rumen-protected L-carnitine, Carneon 20 Rumin-Pro, Kaesler Nutrition GmbH, Cuxhaven, Germany). Then, the proportion of concentrate was increased up to 50% within two weeks *post partum* (*pp*) which was maintained for the rest of the experiment until week eighteen. Water was offered *ad libitum* during the whole experiment. Sixteen weeks *pp*, all cows were intravenously exposed to LPS (0.5 µg/kg live weight, extracted from *Escherichia coli* O111:B4, Sigma Aldrich, St. Louis, Missouri, USA). For the determination of white blood cell (WBC) count, EDTA blood samples were taken frequently 0.5, 1, 2, 3, 4, 6, 9 and 12 h after LPS-injection *via* indwelling catheter (*Vena jugularis externa*) and on day -14, -1, 7 and 14 relative to LPS-injection via venepuncture. WBC were determined using an automatic cell analyser (Celltac MEK 6500 α, Nihon Kohden Europe GmbH, Rosbach, Germany). Statistical analyses were performed using the MIXED procedure of the software package SAS (9.4) with time (as repeated measure), group and their interaction as main factors.

Results: All parameters of the white blood cell count were influenced by LPS, resulting in a significant time-dependent variation ($p_{time}<0.001$). Total leukocyte count decreased significantly 3 h after LPS injection (1.2 G/l) compared to day -14 (6.8 G/l) and returned to initial values after 7 days (7.2 G/l). This progression was also reflected in granulocyte and lymphocyte counts. L-carnitine supplementation resulted in a 20% higher eosinophil count compared to CON over the whole trail period ($p_{group}=0.021$, $p_{time}<0.001$, $p_{group*time}=0.199$).

Conclusions: The present study demonstrated a characteristic LPS induced leukopenia whereas L-carnitine supplementation did not modulate this innate immune response. Supplementation of L-carnitine led to increasing eosinophil granulocyte counts independent of *in vivo* LPS stimulation. This might be due to the stabilising effect of L-carnitine on biological membranes under inflammatory conditions (1). The selective effects of carnitine on eosinophil granulocytes remain to be clarified. Further parameters of cellular energy metabolism, like mitochondrial function, will be assessed to evaluate our hypothesis.

#Name of joint project

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* *Friedrich-Loeffler-Institut Institute of Animal Nutrition, Bundesallee 37, 38116 Braunschweig, Germany, susanne.daniels@fli.de*

113.

MitoCow# - Short-chain acylcarnitine profiles in L-carnitine supplemented Holstein dairy cows challenged by calving and intravenous LPS application

MitoCow# - Profil der kurzkettigen Acylcarnitine in L-Carnitin supplementierten Holstein Milchkühen unter dem Einfluss der Kalbung und einer intravenösen LPS Challenge

*Grindler S., Frahm J., Dänicke S., Huber K. – Stuttgart / Braunschweig

This work is part of the MitoCow project, which aimed to elucidate the functionality of the cow's mitochondria during individual and standardized stress situations, interlinking a broad variety of metabolic, physiologic and genetic data. Metabolite profiling by a metabolomics approach provides novel insights into metabolic pathways associated with mitochondrial functionality of individual dairy cows. The acute adaptation of short chain acylcarnitine profiles to early onset of lactation and to a standardized challenge by intravenous LPS in adult dairy cows has not been determined yet. Huber et. al (2016)[1] hypothesized that higher acylcarnitine concentrations in serum reflected better mitochondrial functions to the adaptive changes after parturition. This capacity to release acylcarnitines into the blood was suggested to be a mechanism to protect mitochondria functionality.

Methods: Blood samples were collected from 59 multiparous German Holstein-Friesian cows during the transition period from late pregnancy to day 126 of lactation. The cows were divided into a control (Con; n=30) and a L-carnitine supplemented group (25 g rumen-protected carnitine/ d/animal; Car; n=29). The dietary carnitine supplementation started on day 42 *ante partum* after the first blood collection. The first individual challenge was set around calving; the second standardized challenge was set at day 111 after calving by intravenous injection of 0.5 µg/kg LM *E. coli* lipopolysaccharides (LPS). For metabolome analysis 10 time points were chosen: day 42 *ante partum* (*a.p.*), 6 h, 12 h, 72 h and day 100 *post partum* (p.p.) 6 h, 12 h, 24 h, 72 h after LPS injection and day 126. Metabolite profiles were determined in EDTA-plasma samples by liquid chromatography and mass spectrometry using the AbsoluteIDQ p180 Kit (Biocrates Life Science AG, Innsbruck, Austria). Metabolite concentrations were given in µmol/L and blood insulin level were given in µg/L. Blood insulin levels were measured by a bovine-specific insulin ELISA (Mercodia AB, Uppsala, Sweden) at day 42, 14 and 3 *a.p.*, 0.5, 1, 2, 3, 4, 6, 9, 12, 24, 48, 72 h *p.p.* and 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 24, 48, 72 h after LPS injection. Data were analysed by two-way ANOVA, $p < 0.05$ was set as significant.

Results: Most prominent, a strong increase was observed in all short-chain acylcarnitines (two-way ANOVA; factor group $p < 0.0001$) acutely after calving and LPS injection in Car group. Sum of long-chain acylcarnitines of Car group was also significantly higher until 72 h p.p.($p < 0.005$). During LPS challenge, Car and Con expressed equal levels. Medium-chain acylcarnitines were not significantly affected by carnitine. Blood insulin concentration decreased from day 14 (0.89 ± 0.04 µg/L; mean ± SEM) a.p. until 1 h (0.11 ± 0.20 µg/L; factor time $p < 0.0001$) p.p. and increased again at 3 h (0.50 ± 0.07 µg/L; factor time $p < 0.0001$)p.p.. Similarly, it was observed shortly after LPS injection, that insulin concentration decreased from day 110 p.p. before LPS injection (0.59 ± 0.07 µg/L) until 1 h (0.20 ± 0.05 µg/L; factor time $p < 0.001$) after injection and increased again at 2 h (0.77 ± 0.09 µg/L; factor time $p < 0.0001$) after injection.

Conclusions: Dietary carnitine supplementation potentially led to a stimulation of mitochondrial functionality as indicated by higher short- chain acylcarnitine concentrations in plasma. In contrast, insulin-glucose metabolism was not affected by dietary carnitine.

#Name of joint project

1) HUBER K., DÄNICKE S., REHAGE J., SAUERWEIN H., OTTO W., ROLLE-KAMPCZY U., VON BERGEN M. (2016): Scientific Reports 6:24642

* *University of Hohenheim Animal Science, Stuttgart, Germany, s.grindler@uni-hohenheim.de*

114.

MitoCow# - Effect of a L-carnitine supplementation on functionality of peripheral blood mononuclear cells (PBMC) in dairy cows challenged by bacterial lipopolysaccherides (LPS)

MitoCow# - Einfluss von einer L-Carnitin Zulage auf die Funktionalität von PBMC in LPS-behandelten Milchkühen

*Daniels S. U., Meyer J., Frahm J., Kersten S., Kluess J., Meyer U., Huber K., Dänicke S. – Braunschweig / Stuttgart

LPS induced systemic inflammation is associated with an increased energy consumption of cows. The efficiency of mitochondrial fatty acid metabolism, a crucial process for the cellular energy supply, depends on an adequate carnitine availability. L-carnitine is essential for fatty acid transport and its supplementation could enhance mitochondrial efficacy and prevent dysfunctions. The aim of the study was to characterize the PBMC mitochondrial respiration profile and proliferation in dairy cows supplemented with L-carnitine and their response to LPS-induced systemic inflammation.

Methods: For the feeding experiment 55 pluriparous German Holstein cows were fed energetically balanced rations with (CAR, n=27) or without (CON, n=27) L-carnitine (25 g/day of rumen-protected L-carnitine, Carneon 20 Rumin-Pro, Kaesler Nutrition GmbH, Cuxhaven, Germany). Experimental feeding started six weeks *ante partum* and lasted until 18 weeks *post partum*.

Heparinised blood samples were taken on day -14, 24 h and day +14 relative to LPS-injection (0.5 µg/kg live weight, *Escherichia coli* O111:B4, Sigma Aldrich, St. Louis, Missouri, USA) which was administered 16 weeks after calving. PBMC were isolated by density gradient centrifugation, seeded (10^6 cells/well) in XF96-well plates (Agilent, Santa Clara, USA) and incubated (1h, 37°C, without CO_2) in Seahorse medium with or without the addition of 1µg/well LPS or 2.5 µg/well ConA. After changing the medium (assay medium with or without additives) the oxygen consumption rate (OCR) was measured under basal conditions followed by the sequential addition of oligomycin, carbonyl cyanide-p-(trifluoromethoxy) phenylhydrazone (FCCP) and rotenone/antimycin A using Seahorse XFe96 instrument (Agilent). Furthermore, a proliferation assay was performed with non-stimulated or ConA stimulated PBMC ($1x10^5$ cells/well) in 96-well plates and incubated for 69.5 h (37°C, 5 % CO_2). Fluorescence was measured (Excitation: 540 nm, Emission: 590 nm) with plate reader Infinite 200 Pro (Tecan Group Ltd., Männedorf, Switzerland) 2.5 h after addition of Alamar Blue. Statistical analyses were performed using the MIXED procedure of the software package SAS (9.4) with time relative to *in vivo* LPS (as repeated measure), group, *ex vivo* stimulus and their interaction as main factors.

Results: Parameters for basal respiration, proton leak, maximal respiration, spare respiratory capacity and ATP-production were unaffected by L-carnitine supplementation. In response to LPS injection proton leak and ATP-production of all cows showed decreased OCR, basal and maximal respiration showed increased OCR. All cows showed 34% higher stimulation index of proliferation assay in response of *in vivo* LPS. In turn, e*x vivo* LPS administration significantly reduced OCR in both feeding groups. Initially (day -14), the PBMC non-mitochondrial respiration rate of L-carnitine supplemented cows, independent of *ex vivo* stimulus, was 13.4% lower than that of cows of the control group, but significant 12.5% higher 14 days after LPS-injection.

Conclusions: Present data show that intravenous LPS administration increased non-mitochondrial respiration rate in PBMC of L-carnitine supplemented dairy cows, indicating a higher non-mitochondrial enzymatic activity in response of LPS in these cows. Increased stimulation index after *in vivo* LPS administration indicates a higher proliferation rate.

#Name of joint project

* *Friedrich-Loeffler-Institut Institute of Animal Nutrition, Bundesallee 37, 38116 Braunschweig, Germany, susanne.daniels@fli.de*

115.

Effect of rumen cannulation in young calves on performance and immune response to rumen content

Einfluss der Pansenfistulierung bei jungen Kälbern auf Wachstum, Futteraufnahme und Immunantwort gegen Panseninhalt

*Tümmler L.-M., Vernunft A., Kuhla B. – Dummerstorf

Ruminal cannulation is frequently used to study ruminal digestion and physiology. It has been reported to have no adverse short-term effects on the performance of young calves (1). To our knowledge there is no information available on long-term effects. Therefore, the objective of the present analysis was to evaluate the longer-term effects of rumen cannulation on performance and furthermore on the immune response to rumen content in young calves.
Methods: Female newborn Holstein calves (n=28) were studied from birth to the age of 23 weeks (wk). Calves were allocated to 2 groups, either rumen cannulated (CAN, n=14) or non-cannulated (NON, n=14). CAN calves were equipped with a rumen cannula in wk 3. NON calves did not undergo sham surgery. Additionally, half of the NON and CAN group was either fed with 10% (10%-MR, n=14) or 20% (20%-MR, n=14) of their body weight (BW) with milk replacer (MR) after a 2.5 days period of colostrum feeding. MR feeding of the 20%-MR group was reduced to 10% of BW from wk 9 to the end of wk 10. Both groups were weaned from wk 11 to the end of wk 12. Further feeding regime was identical for all animals. Hay was fed for ad libitum intake until the end of wk 14. Starter was fed for ad libitum intake until the end of wk 12 and subsequent intake was restricted and reduced until the end of wk 16. A total mixed ration was offered for ad libitum intake from wk 11 onwards. Feed intake was recorded daily and BW weekly. Serum samples, collected in wk 7 (42.7 d ± 0.4), were analyzed by Western Blot for antibodies against proteins derived from body-own rumen fluid or a pool of rumen fluids from all calves. For statistical analysis an ANOVA was performed using SAS software with time as repeated variable, if applicable. The model contained the fixed factors cannula (CAN, NON), feeding (10%-MR, 20%-MR), time (if applicable) and all interactions. For analysis of pairwise differences the Tukey-Kramer procedure was used.
Results: Dry matter intake (DMI) of MR was less in CAN calves in wk 6-11 ($P < 0.05$). Cannulation had no effect on DMI of hay. DMI of starter, however, was higher in wk 12-15 ($P < 0.05$), whereas DMI of TMR tended to be lower in wk 12, 13, 21 and 22 ($P < 0.1$) and was lower in wk 14 and 20 ($P < 0.05$) in CAN calves. Total DMI of solid feed was affected by cannulation only in wk 20-22 with lower levels in CAN calves ($P < 0.05$). BW of CAN calves tended to be lower in wk 11 and 12 ($P < 0.1$) and continued to be lower thereafter, until wk 23 ($P < 0.05$). Antibody levels against proteins from body-own rumen fluid were higher in CAN calves ($P < 0.05$), whereas levels against proteins from the rumen fluid pool did not differ between cannulation groups.
Conclusions: The results indicate that rumen cannulation affects performance of young calves with some delay. In the longer term, rumen cannulation can persistently impair BW, alter feed preferences and increase antibody titers against body-own ruminal proteins. As the control group received no sham surgery, the effect of the cannula itself cannot be distinguished from the effect of the surgical procedure.
Acknowledgement: This study is part of the JPI FACCE program and financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE) (2814ERA04A).

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* *Leibniz Institute for Farm Animal Biology (FBN) Institute of Nutritional Physiology, Wilhelm-Stahl-Allee 1, 18196 Dummerstorf, Germany, tuemmler@fbn-dummerstorf.de*

116.

Buccal swabs as a non-invasive sampling method to monitor the rumen microbiota in calves

Mundschleimhautabstriche als nicht-invasive Beprobungsmethode zur Beschreibung der ruminalen Mikrobiota in Kälbern

*Amin N., Kinoshita A., Tröscher-Mußotter J., Schwarzkopf S., Dänicke S., Camarinha-Silva A., Huber K., Frahm J., Seifert J. – Stuttgart / Braunschweig

The analysis of the rumen microbiota is highly dependent on the accessibility of the rumen content which is limited to invasive methods like stomach tubing or rumen fistulas. The oral mucosa and saliva of ruminants are expected to include rumen microbes due to regurgitating of rumen contents by rumination. Thus, sampling saliva could probably be an adequate strategy to characterize parts of the rumen microbiota in the mouth of ruminants. A proof of concept was done using buccal swabs to collect microbial biomass in saliva of dairy calves from the first week to around 150 days postpartum.

Methods: Female German Holstein calves (n = 59) were studied from day of birth until day 149 ± 10 of life. Calves were fed twice daily with three liters of pooled herd milk and milk replacer (MR) from day 5 – 12 after birth. Study started at a mean age of 8 ± 1.9 days and 44.5 ± 5.2 kg of body weight. They were randomly and evenly assigned to either an early weaning (earlyC) or a late weaning (lateC) group. Both groups were fed with 1.35 kg MR/d available at a maximum volume of 9 liters per day. Over the entire trial, all calves received hay and water ad libitum and had access to a maximum of 2 kg concentrate per day until weaning. Offered amount of concentrate was reduced to 1 kg/d after weaning for earlyC (day 42) or during weaning for lateC (day 98). During weaning, the milk replacer was gradually reduced by 0.09 kg/d within 14 days. Post-weaning, at experimental day 42 (earlyC) or day 112 (lateC) calves received a total mixed ration consisting of 48 % grass silage, 32 % maize silage and 20 % concentrate. Rumen samples were collected at day 140 by stomach tubing. Saliva samples were collected using sterile cotton wool swabs which were placed on a clamp in the mouth at day 1, 28, 42, 70, 98, 112, and 140 of the experimental periods for about 30 seconds. Bacterial cells were eluted from the swabs by mixing them with PBS buffer and sonication. DNA from the swab cell suspension and rumen fluid was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedical). Illumina sequencing of the 16S rRNA gene was used to characterize the overall bacterial diversity [1]. The bioinformatic analysis of sequencing data was done using QIIME 2 pipeline and for taxonomic annotation RDP database was used. Multivariate statistical analyses were done using PRIMER-E and Calypso [1, 2].

Results: 16S rRNA gene sequencing resulted in a total of 4877 operational taxonomic units (OTUs) in saliva samples of all time points and 2401 OTUs in rumen fluid samples at day 140. The total number of OTUs in saliva samples increased from 1103 to 4415 OTUs (day 1-140) indicating an evolution of the salivary community and the influence of the appearing rumen microbiota during the development of the calves. This was approved by comparing the number of OTUs shared between saliva and rumen fluid changing from 22% at day 1 to 53% at day 140. The core rumen microbiota was defined based on this dataset and can be clearly distinguished from the salivary community. Comparison of weaning groups showed higher bacterial diversity in the salivary communities of earlyC compared to lateC at day 70 and day 98.

Conclusions: The data showed the establishment of rumen microbiota during the development of calves as defined by a core microbiota among all time points analyzed. The present study indicated the applicability of buccal swabs to monitor the rumen microbiota along a critical period of calf life (i.e. weaning), which is a challenging phase for the development of calves, the establishment of the rumen and its microbiota.

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* *University of Hohenheim Institute of Animal Science, Stuttgart, Germany, nidaamin786@gmail.com*

117.

Effect of wilting grass silage on *in situ* ruminaldegradation of crude protein and organic matter

Einfluss des Anwelkgrades von Grassilage auf den ruminalen Abbau von Rohprotein und organischer Masse in situ

*Rupp C., Westreicher-Kristen E., Susenbeth A. – Kiel / Lelystad

Research has shown a positive effect of wilting on the amount of *rumen undegraded crude protein (RUCP)* in silages (1). But few information is available about the effect of wilting on the extent of organic matter (OM) degradation, which acts as the main determinant for microbial crude protein (MCP) yield. Therefore, both factors affect total *post ruminal crude protein (prCP)*, the sum of RUCP and MCP. Due to their high daily intake, even a small enhancement of the amount of prCP in roughages provides a significant contribution to meet the protein demand in ruminants. The aim of this study was to investigate the effect of wilting intensity on prCP concentration in grass silage.

Methods: An intensively managed grass land sward– predominantly consisting of perennial ryegrass (*Lolium perenne*) - from the second cut in 2017 was used. After harvest, the grass was wilted to realize eight different dry matter (DM) concentrations (170, 310, 390, 420, 470, 530, 580 and 600 g DM/ kg fresh matter, respectively). Upon reaching the respective DM, the grass (15 % CP in DM) was chopped and ensiled in quadruplicate (control, CON), whereby half of the material of the respective DM concentration was treated with a silage additive containing lactic acid bacteria (LAB; BonsilagePlus, Co. Schaumann, Germany). Subsequently grass was pressed in 1.5 L glass jars according to the scheme for silage testing in Germany (2) and stored for 90 days. Silage samples were freeze-dried and ground to pass a 2 mm sieve size. About 1.5 g material was packed in nylon bags for *in situ* incubation using three ruminally fistulated Jersey cows. The cows received a total mixed ration composed of (per kg DM) 350 g maize silage, 350 g grass silage, 250 g hay, 30 g barley straw and 20 g of mineral mixture. The treatments are referred as treatment-DM concentration at ensiling, e.g. LAB-170 or CON-170. Two replicates per treatment and time point were incubated in each cow for 2, 4, 8, 16, 24, 48, and 72 h. Original samples and incubation residues were analysed for CP and OM concentration. All residuals were corrected for particle losses. Residual CP was corrected for microbial contamination according to Krawielitzki *et al.(*2006) (3). The exponential model of Ørskov and McDonald (1979) was fitted to the data with consideration of Lag-Time. Effective degradability was calculated assuming a passage rate of 0.04/h. Ruminal MCP synthesis was assumed to be 181 g per kg fermented OM (4). The concentration of prCP was calculated by the sum of RUCP and MCP.

Results: $RUCP_{0.04}$ (g/kg CP) showed a quadratic response ($p<0.001$) to increasing DM concentrations for both treatments, with lowest values for CON-420 (106 g) and LAB-470 (110 g). LAB treatment resulted in a higher content of $RUCP_{0.04}$ compared to CON treatment (means for all DM concentrations, CON: 110, LAB: 115 g/kg CP; $p<0.001$). There was a quadratic effect ($p<0.001$) of DM concentration on estimated $MCP_{0.04}$ yield (g/kg OM) caused by a lower OM degradation at DM ranges between 390-530 g/kg, without any difference among both treatments (CON: 117.4, LAB: 116.6 g; $p=0.76$). The concentration of $prCP_{0.04}$ also showed a quadratic relationship ($p<0.001$) for both treatments, with lowest values at DM contents between 390-530 g/kg. There was no effect of LAB on $prCP_{0.04}$ concentration (CON: 136.0, LAB: 136.4 g/kg OM, $p= 0.48$).

Conclusions: The enhancement of $RUCP_{0.04}$ through wilting as reported in the literature can be confirmed by our results. However, this positive effect was largely compensated by the reduced OM degradation resulting in lower MCP yield.

Acknowledgment: We gratefully acknowledge the Institute of Animal Science (Dept. of Animal Nutrition) of Hohenheim University for providing the facility for performing the *in situ* trials and the Chamber of Agriculture (Schleswig-Holstein) for the experimental material.

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* *Christian-Albrechts-Universität zu Kiel Institute of Animal Nutrition and Physiology, Hermann-Rodewald-Str. 9, 24118 Kiel, Germany, rupp@aninut.uni-kiel.de*

118.

Effect of phytogenic substances on saliva composition and production and ruminal pH in dry cows

Einfluss von phytogenen Substanzen auf die Speichelsekretion, Speichelzusammensetzung und den Pansen pH-Wert in trockenstehenden Kühen

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

Subacute ruminal acidosis is still a major health problem in cattle fed high-grain diets. Saliva modulates ruminal pH, but saliva production decreases in cattle fed high-grain diets. Strategies to increase saliva production or improve composition and its impact on ruminal pH are limited. Therefore, this study was conducted to screen different phytogenic substances and their impact on salivary production and composition, rumination and ruminal pH in dairy cattle fed high-grain diets.

Methods: Nine non lactating ruminally cannulated Holstein cows were transitioned to a ration containing 65% of grain. Nine phytogenic compounds, each at two dosages, were evaluated in four cows each using a change-over design balanced for carry-over effects: angelica root (6.6 and 66 ppm), capsaicin (10 and 100 ppm), gentian root (6.6 and 66 ppm), garlic oil (0.3 and 3 ppm), ginger (40 and 400 ppm), L-menthol (6.7 and 67 ppm), mint oil (15.3 and 153 ppm), thyme oil (9.4 and 94 ppm) and thymol (5 and 50 ppm). Each plant compound was compared to a control containing silica wheat carrier. The treatment was mixed with 50 g of carrier, then blended with 2.5 kg DM of feed and offered to cows in the feed bunk. Animals were restricted from feed for 12 hours prior to feeding. Saliva was collected using a vacuum pump before and 4 hours after treatment. Saliva was analyzed for bicarbonate, phosphate, total proteins, buffer capacity, and lysozyme activity. Saliva flow to rumen (g/min) and feed ensalivation (g/g DM) were evaluated by DM analysis of feed boli collected from the cardia over a 30-min period. Rumination was monitored for 10 h following treatment, and ruminal pH was also measured during this period at 15 minute intervals. Data were analysed with the Proc Mixed procedure of SAS with dose as fixed effects and cow as random effect, linear and quadratic effects were evaluated. Data on saliva composition prior to treatment were used as a covariate; measurements of boli from the same cow within treatment were analyzed as repeated measures.

Results: Saliva flow linearly increased with gentian root ($P < 0.01$; 143, 169 and 212 ± 19.4 g/min), ginger ($P < 0.05$; 133, 182 and 181 ± 17.7 g/min) and menthol ($P < 0.05$; 114, 143 and 147 ± 20.5 g/min) for control, low and high dose, respectively. Feed ensalivation increased with capsaicin ($P < 0.01$; 5.1, 4.2 and 7.4 ± 1.06 g/g feed DM) and L-menthol ($P < 0.05$; 4.9, 5.9 and 6.5 ± 1.15 g/g feed DM) for control, low and high dose, respectively. Saliva buffer capacity displayed a linear increment with both garlic oil ($P < 0.05$; 0.015, 0.016 and 0.018 ± 0.0011 mol of HCl/L/ΔpH) and with thyme oil ($P < 0.05$; 0.013, 0.015 and 0.018 ± 0.0013 mol of HCl/L/ΔpH) for control, low and high dose, respectively. Saliva bicarbonate increased with garlic oil ($P < 0.05$; with estimates of 67.0, 93.3 and 97.2 ± 7.40 mmol/L for control, low and high dose, respectively. No effects were found on saliva phosphate ($P \geq 0.12$), total proteins ($P \geq 0.15$) and lyzozyme activity($P \geq 0.14$). Angelica root tended to increase rumination time ($P = 0.07$; 27, 41 and 42 ± 5.9 min) for control, low dose and high dose, respectively. L-menthol had a quadratic effect on mean ruminal pH ($P < 0.05$; 6.0, 6.3 and 6.1 ± 0.07), the time that ruminal pH < 6.2 ($P < 0.05$; 153, 71 and 150 ± 25.4 min), and on acidosis index post-treatment ($P < 0.05$; 21.4, 5.9 and 13.8 ± 3.37 min/kg DM) for control, low and high dose, respectively. Furthermore, thyme oil tended to linearly decrease the area that pH < 6.2 post-treatment ($P = 0.10$; 65.7, 63.0 and 19.4 ± 17.57 pH × min) for control, low and high dose, respectively.

Conclusions: Phytogenic substances displayed a positive effect on saliva production and composition, rumination or ruminal pH; therefore, they may have the potential to alleviate subacute ruminal acidosis when fed to dairy cows as part of a high-grain diet.

** Vetmeduni Wien Tierernährung und Funktionelle Pflanzenstoffe, Vienna, Austria, ezequias.castillo-lopez@vetmeduni.ac.at*

119.

Impact of physically effective fibre at different dietary rumen nitrogen balance on chewing behaviour, nitrogen balance, and performance of lactating dairy cows

Einfluss des physikalisch effektiven Fasergehaltes in Abhängigkeit der ruminalen Stickstoffbilanz auf Kauverhalten, Stickstoffbilanz und Leistung von laktierenden Milchkühen

*Heering R., Selje-Aßmann N., Dickhoefer U. – Stuttgart

The aim was to analyse the effects of dietary physically effective fibre (peNDF) concentration depending on the rumen nitrogen balance (RNB) on chewing behaviour, performance, and partitioning in nitrogen (N) excretion in lactating dairy cows. It was hypothesized that greater dietary peNDF concentrations increase N recycling via intensified chewing activity, and thus enhance the efficiency of N use. This would promote rumen microbial protein synthesis and shift partitioning of N towards faeces and milk, with more pronounced effects at reduced RNB level, as microbes will recycle unused N in the blood.

Methods: Twenty lactating Holstein cows with an average (mean ± standard deviation) milk yield of 39 ± 7 kg/day and 103 ± 59 days in milk were assigned to a 4 x 4 Latin Square with four periods of 13 days adaptation and 8 days of sampling. Treatments were RNB level and peNDF concentration. The RNB levels were adjusted by varying the ingredients of a concentrate mixture: balanced RNB (RNB0; 0.1 g N/kg DM; 2.6 g N/day) and negative RNB (RNB-; -1.5 g N/kg DM; -39 g N/day). Dietary peNDF concentrations were solely varied by adjusting the particle size via the mixing time of the diet: 28 min (high peNDF) and 58 min (low peNDF). Diets were formulated to be isoenergetic and offered *ad libitum* as a total mixed ration with maize silage and grass silage as main roughage components (forage to concentrate ratio of 57:43 on dry matter (DM) basis). Titanium dioxide (TiO_2) was used to estimate daily faecal excretion. Daily feed intake and milk yield were recorded. Feed and milk samples were taken and analysed for their chemical composition. Dietary particle size distributions were determined using the Penn State Particle Separator with three sieves (19, 8, and 4 mm) and used to calculate peNDF concentrations. Chewing behaviour of cows was recorded using noseband pressure sensors (RumiWatch, Itin+Hoch GmbH, Liestal, Switzerland). Rectal faecal grab samples were taken and analysed for DM, N, and TiO_2, and urine spot samples for N. Urinary N excretion was estimated as the difference between daily N intake of individual cows and N losses via milk, faeces, skin, and hair. Data were analysed using PROC MIXED in SAS V9.4 with peNDF and RNB level, period, and their interactions as main effects and animal within group as a random factor. Days in milk was used a covariable. Significance level was $P<0.05$.

Results: The peNDF concentrations (particles > 8 mm) were 21.7 g and 20.7 g/100 g DM across RNB levels ($P<0.01$). High peNDF concentration tended to prolong chewing time ($P=0.06$). However, an interaction showed that high peNDF concentration decreased chewing time when cows were fed a RNB0 diet, but increased it when fed a RNB- diet ($P<0.01$). Greater peNDF concentration resulted in a higher number of total chews regardless of RNB level (<0.01) and this increase was more pronounced at RNB-. Daily DM intake ($P<0.01$) and energy-corrected milk yield ($P=0.06$) were lower with high than with low peNDF concentrations. Milk urea-N content was lower at RNB- diets ($P<0.01$), but similar for both peNDF levels ($P=0.56$). Milk N use efficiency was higher in RNB- compared to RNB0 diets and in high peNDF compared to low peNDF diets ($P<0.01$). Partitioning of N shifted from urine towards faeces and milk at RNB- ($P<0.01$), but was not affected by peNDF concentration ($P\geq0.38$). The positive effect of peNDF concentration on the partitioning of N was more pronounced at RNB- ($P\leq0.03$).

Conclusions: Elevating peNDF concentration can increase milk N use efficiency of cows, however, decreases feed intake and animal performance. Feeding cows a diet with a high peNDF concentration shifts N partitioning with more pronounced effects at low compared to balanced RNB.

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* *University of Hohenheim Animal Nutrition and Rangeland Management in the Tropics and Subtropics, Fruwirthstr. 31, 70599 Stuttgart, Germany, ruth.heering@uni-hohenheim.de*

120.

Dynamics of salivary composition of dairy cows fed high grain diets

Veränderungen der Speichelzusammensetzung von Holsteinkühen nach einer hohen Kraftfuttergabe in der Ration

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

Diet is a well-known factor impacting ruminal dysbiosis. However, diet and dietary intake both impact other physiological factors related to ruminal dysbiosis, including saliva production. Saliva is an essential buffer that helps to modulate ruminal pH by providing bicarbonate to the rumen, but saliva production decreases in cattle fed high-grain diets. Therefore, in order to better understand the process of ruminal dysbiosis, this study investigated the effects of high grain feeding on the salivary composition and secretion directly after adaptation to a high grain diet and after two weeks of high grain feeding.

Methods: Nine ruminally cannulated non-lactating Holstein cows were transitioned to a 65% concentrate ration (wheat/triticale/bakery-bread product) 45% roughage (corn and grass silage) and blocked into one of two sampling groups. Sampling occurred on day 2 after a seven day gradual (10% daily increase) adaptation to the high grain diet, and again on day 20 after adaptation to the diet. Prior to sampling, cows were restricted from feed for 12 h. Saliva samples for composition were collected orally using a vacuum pump at 0 h and 4 h relative to morning feeding. Salivary flow was measured by the collection of feed boli from the cardia during the morning meal over a 30 minute sampling period. Saliva from the boli was collected using gauze to filter out feed particles. Saliva flow was measured as total saliva weight collected per minute of sampling (g/min) and ensalivation of the bolus was calculated as the weigh of saliva per gram of feed (g/g DM). Saliva pH was measured immediately after collection. Osmolality, buffer capacity, lysozyme activity, as well as bicarbonate, phosphate, and total protein concentrations were measured in the lab using various commercial kits. Data were analysed with the Proc Mixed procedure of SAS with day, time, and the interaction as fixed effects, group as random effect and cow as a repeated measure.

Results: Acidosis index (1) was significantly higher on day 20 compared to day 2 (5.17 vs. 1.87 Area under the curve at pH 6/kg DMI, respectively). Salivary pH, buffer capacity, and the concentrations of bicarbonate, phosphate and total protein did not change as a result of a feeding event nor due to 20 days of high grain feeding. Osmolality was significantly higher ($P = 0.01$) at day 20 of high grain feeding compared to day 2 (253.06 vs. 237.17 mOsm/kg, respectively), but was not affected by a feeding event. Lysozyme activity of the saliva tended to be lower at 4 h after a feeding event ($P = 0.07$; 49.54 vs. 35.24 U/mL/min, respectively). No effect of interaction was seen between day and time for any of the measured variables. Ensalivation of the bolus was significantly higher ($P = 0.03$) on day 2 compared to day 20 of high grain feeding (5.06 vs. 4.32 g/g of DM, respectively), whereas saliva flow was higher on day 20 compared to day 2 of high grain feeding ($P = 0.04$; 140.1 vs. 110.5 g/ min, respectively). Between boli collected within a feeding period, there was a both a significant effect of saliva flow ($P = 0.001$) as well as a significant effect of ensalivation ($P = 0.01$). Furthermore, a tendency towards an interaction was seen between bolus collected and the day of sampling for both ensalivation ($P = 0.07$) and saliva flow ($P = 0.10$).

Conclusions: Salivary composition of the measured variables tended to remain stable with long term exposure to high grain diets. The osmolality increased on day 20, indicating salivary osmolyte changes compared with day 2. Also, the salivary secretion rates changed from day 2 to 20 with ensalivation per bolus decreasing, which likely led to an increased severity of acidosis with duration of high grain challenge, as indicated by the increased acidosis index on day 20.

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* *Vetmeduni Wien Tierernährung und Funktionelle Pflanzenstoffe, Veterinärplatz1, 1210 Vienna, Austria, renee.petri@vetmeduni.ac.at*

121.

Evaluation of fecal fermentation pattern and the fecal bacterial community in dairy cows fed organically produced forage-based TMR with normal and reduced particle size

Charakterisierung des Mikrobioms sowie der Fermentationsprodukte im Kot von Milchkühen, die mit einer grundfutterbasierten Ration mit reduzierter Partikelgröße gefüttert wurden

*Castillo-Lopez E., Haselmann A., Petri R. M., Knaus W., Zebeli Q. – Vienna

Utilization of forages is essential in dairy cattle, especially in organic dairy farming, fed forage-rich diets. Reduction of particle size could improve feed intake and nutrient degradation in the rumen, and hence forage utilization. However, this may lead to an increased nutrient flow to the hindgut, which may influence the microbial population and the associated metabolic activities. The objective of this study was to evaluate the effect of reduced dietary forage particle size on fecal pH, fecal short chain fatty acids (SCFA), and the fecal bacterial community structure in organically fed dairy cows consuming forage-based rations.

Methods: Twenty-one lactating Holstein cows (4 primiparous and 17 multiparous; mean and SD, 703 ± 65 kg BW and 135 ± 104 DIM) were divided in two groups and were fed 1 of 2 diets for 34 d. Diets contained 20% concentrate, and 80% forage (DM basis), and were fed as either a control (CON) with a physically effective $NDF_{>8}$ content of 36.9% (11 cows), or in a reduced particle size (RED) with a physically effective $NDF_{>8}$ content of 21.2% (10 cows). The proportion of long size particles (>19 mm) was 74 and 23%, medium size particles (8-19 mm) was 10 and 26%, short size particles (1.18-8 mm) was 12 and 37%, and the proportion of small size particles (<1.18 mm) was 5 and 14%, for CON and RED, respectively. The forage portion of the TMR was composed of 43% grass hay and 37% clover-grass silage, and the concentrate included 40% field peas, 40% sunflower cake, 15% wheat bran, and 5% vitamin and mineral premix (DM basis). Fecal samples were collected every 8 hours within the last 3 d of the experiment, fecal pH was immediately measured and then subsamples were frozen at -20 °C. At the end of the experiment, samples were thawed and analyzed for SCFA with gas chromatography. DNA was extracted from fecal samples to evaluate the bacterial community using a 16S rRNA Illumina sequencing platform and bioinformatics. Data were analyzed with the Proc Mixed procedure of SAS with treatment as fixed effect and cow as random effect. Measurements of fecal pH from the same cow were analyzed as repeated measures. Correlation analysis between SCFA concentration and bacterial taxa was conducted.

Results: Fecal pH was lower for cows consuming the RED diet ($P < 0.05$) with estimates of 7.24 and 7.16 ± 0.027 for CON and RED, respectively. The concentration of total SCFAs was not affected by particle size and averaged 56.52 ± 4.23 μMol/g, but the percentage of propionate tended ($P = 0.08$) to be greater for cows fed the RED diet (13.30 and 13.76 ± 0.17%). Bacterial diversity was not affected ($P = 0.34$) as indicated by number of observed OTUs with an average of 1045 ± 18.3 OTUs. Proportions of predominant bacterial phyla, including *Firmicutes* (58.03%), *Bacteroidetes* (27.14%), *Verrucomicrobia* (4.04%), and *Lentisphaera* (2.09%)were not affected ($P \geq 0.11$) by particle size. Relative abundance of *Lachnospiraceae* was increased ($P = 0.05$) in cows fed RED diet compared to the CON (12.17 and 13.95 ± 0.590%). Low abundant fecal genera tended to show the most changes in relation to particle size. *Acetitomaculum,Turicibacter*, *Ruminobacter*, and *Anaerosporobacter* genera increased ($P < 0.05$) when the particle size of the forage was reduced. Relative abundance of some bacterial taxa was correlated with major SCFA concentration. For example, *Ruminococcaceae* was positively ($P < 0.05$) correlated with acetate, but negatively correlated ($P < 0.05$) with propionate concentration.

Conclusions: Reducing forage particle size lowered fecal pH without affecting the SCFA concentration, and tended to enhance propionate while maintaining the fecal bacterial diversity.

* *University of Veterinary Medicine Vienna Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, Vienna, Austria, ezequias.castillo-lopez@vetmeduni.ac.at*

122.

First insight into the mode of action of menthol regulating the transcriptome of ruminal epithelium and modulating the rumen microbiota in sheep

Erste Einblicke in die Wirkmechanismen der Transkriptom-Regulation im Pansengewebe und die Modulierung der Pansenmikrobiota durch Menthol im Schaf

*Schulte J. N., Patra A. K., Park T., Geiger S., Yu Z., Schrapers K. T., Aschenbach J. R. – Berlin / Columbus / Kolkata

Advancements in RNA sequencing facilitate new insights into effects of PBLC (plant bioactive lipid compounds) on a cellular level. We applied MACE-Seq (Massive Analysis of cDNA Ends) to identify differentially expressed genes (DEG) in the rumen of sheep after long-term dietary supplementation of menthol. Especially in the context of calcium absorption, which has been shown to increase through activation of TRP (transient receptor potential) channels via menthol, we aimed to elucidate the modulation of gene expression by prolonged elevation of intracellular calcium uptake. Additionally, we investigated the effects on ruminal fermentation and microbial community composition to obtain a more comprehensive view on the overall effects of menthol in the rumen. Therefore, this study sought to improve our understanding of the mode-of-action and the impact on the ruminal microbiota and epithelium by long-term dietary supplementation of menthol in sheep.

Methods: Twenty-four growing Suffolk sheep were dived into 3 groups and fed600 g concentrate feed without (Control) or with two doses of a menthol-rich feed additive (PBLC-L: 80 mg/d, PBLC-H: 160 mg/d) and hay *ad libitum* for 28 days. Ruminal tissue was analyzed via MACE-Seq for changes in gene expression. Raw reads were mapped and annotated to the ENSMBL genome version 3.1 of *Ovis aries*. Raw reads were normalized according to the geometric mean. *P*-values for the likelihood of DEG and false discovery rate were calculated using DESeq2. Differences of microbiota from solid and liquid digesta fractions were determined using a 16S rRNA metagenomic sequencing approach. Amplicon libraries were analyzed with QIIME2 and quality filtered with DADA2. For taxonomic diversity analysis of ruminal fluid, amplicon sequence variants (ASV) were classified using the Greengenes 99% OTUs (operational taxonomic unit) 16S rRNA gene reference sequences. Relative abundance was analyzed with the mixed model procedure of SAS (2001). Pearson correlations between SCFA and major OTUs were calculated also using SAS. Additionally, chemical composition of ruminal fluid was analyzed to investigate effects on fermentation characteristics. Statistical significance was set at $P \leq 0.05$, whereas a trend was considered at $0.05 \leq P \leq 0.10$.

Results: Transcriptomic analysis revealed changes in gene expression caused by menthol supplementation. For PBLC-L and PBLC-H, 59 common DEGs vs. control were detected ($P < 0.05$), of which 9 genes were up- and 12 genes were down-regulated ($1 < \text{Log2FC} < -1$). Many of the overlapping genes (e.g. FOXS1, MXD1, SLC38A7) are involved in transcriptional regulation, cell homeostasis, or gastrointestinal immunity. For rumen microbiota, phylogenetic diversity increased linearly with PBLC doses ($P = 0.028$). In the solid fraction, evenness ($P = 0.001$) and Simpson diversity index ($P = 0.006$) were quadratically changed by PBLC. Furthermore, microbiota composition was influenced by PBLC, shown by 5 (Control), 7 (PBLC-L), and 11 (PBLC-H) unique OTU's as opposed to 104 shared species-level OTUs in the solid fraction, whereas the liquid fraction showed 93 shared OTUs with 7, 11, and 6 OTUs unique to the treatments. Ruminal fermentation parameters (total SCFA concentrations, pH and proportions of acetate, propionate and butyrate) were not affected by PBLC supplementation.

Conclusions: Our study provides first insights into the effects of menthol on the ruminal epithelium and microbiome of sheep. The dietary supplementation slightly alters the diversity of the microbiota while maintaining a stable fermentation, indicating metabolic redundancy of the ruminal ecosystem. Additionally, overall changes in gene expression of the ruminal tissue point towards a modulation of cell cycle progression, cytoskeleton, and immune response. Therefore, we hereby provide novel candidates by which ruminal epithelial cells respond to a continuous calcium uptake caused by the activation of calcium channel (most likely TRPV3) via menthol.

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* *PerformaNat GmbH, Hohentwielsteig 6, 14163 Berlin, Germany, j.schulte@fu-berlin.de*

123.

Evaluation of three feed additives using an in vitro model for subacute rumen acidosis

Evaluation von drei Ergänzungsfuttermitteln mittels eines In-vitro-Modells zur Simulation einer subakuten Pansenazidose

*Hasselbring L., Eger M., Breves G. – Hanover

Subacute rumen acidosis (SARA) is characterized by a decrease in pH below 5.6 or 5.8 for several hours per day. The prevalence of SARA in dairy herds is high and this may lead to reduced animal welfare and economic losses for the farmer. Therefore, SARA prevention or treatment strategies are a major topic of current research. Recently we established an *in vitro* model for subacute rumen acidosis (1) using the rumen simulation technique (Rusitec). Here we used this model to evaluate the ability of three feed supplements to alleviate SARA conditions.

Methods: The rumen simulation technique was applied using 12 fermentation vessels. The experiment started with a 7 d equilibration period. At day 8, SARA was induced by reducing the concentrations of bicarbonate (20 mmol/L) and phosphate (10 mmol/L) in the buffer solution (induction period, IP). Starting at day 11, the 12 fermenters were assigned to four different treatment groups, receiving either one of the three feed supplements (A1, A2, A3) dissolved in water, or the same amount of water for the control group (CON). All supplements contained living yeast (*S. cerevisiae*). However, while supplement A1 was rich in buffering components such as sodium bicarbonate, calcium carbonate and magnesium oxide, supplements A2 and A3 enclosed lower amounts of carbonate, but additionally contained propionate and phosphate salts. Supplement A2 differed from A3 only by addition of gentian root. From day 11 to day 13, 1.7 g of each compound dissolved in 10 ml water were applied (experimental period 1, EP1). From day 14 until day 20, 2.5 g of each compound dissolved in 12.5 ml water were applied (experimental period 2, EP2). During IP und both experimental periods, pH and redox potentials were measured once per day before exchange of the feed bag. Production rates and molar proportions of short-chain fatty acids and the concentrations of NH_3-N in the effluent were measured every second day. Statistical analysis was performed using the software GraphPad Prism 8. Repeated measurements two-way ANOVA was applied to detect significant effects of the factors time or treatment or interactions of time x treatment. In case of significant effects Tukey post-test was applied.

Results: Induction of SARA led to a decrease in pH to 5.66 ± 0.05 (mean ± SD) for all fermenters at day 11. Thereafter, application of A1 increased pH values compared to the control group for days 14 to 18 and day 20, while addition of A2 and A3 increased pH values at days 15 and 17 ($P < 0.05$). The redox potential increased with decreasing pH, however, stabilized with application of all three supplements on a more negative level compared to the control group ($P < 0.05$). The concentration of NH_3-N was elevated in A2 and A3 treated vessels compared to CON group and A1 group for days 14 to 20 ($P < 0.05$). Total short-chain fatty acid production was higher compared to the control group for A2 and A3 group at days 16 to 20, while for A1 group it was only elevated at day 18 ($P < 0.05$). This effect was mainly based on higher levels of propionate in A2 and A3 group ($P < 0.05$). Moreover, all three supplementary feeds increased the production rate of acetate compared to the control group at days 16 to 20 ($P < 0.05$, except for A2 at day 18). This led to a reduction of the molar proportion of acetate in A2 and A3 group compared to the control group and to an increased proportion of acetate in A1 group towards the end of the experiment ($P < 0.05$). In contrast, the proportion of propionate was increased in A2 and A3 group from day 12 onwards ($P < 0.05$). The molar proportion of isovalerate was lower in A3 group compared to control from days 14 to 20 ($P < 0.05$).

Conclusions: All three supplements were effective in increasing pH values during subacute acidosis in the Rusitec system. While A1 had the highest impact on pH values, A2 and A3 might be used in an energy-deficient situation for the cow due to the high availability of propionate.

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* *University of Veterinary Medicine Hannover Institute for Physiology and Cell Biology, Bischofsholer Damm 15, Geb. 102, 30173 Hanover, Germany, louisa.hasselbring@gmx.de*

124.

Partitioning of degraded organic matter into short chain fatty acids and microbial biomass – a preliminary study on grass- and corn silages

Aufteilung der abgebauten organischen Substanz in kurzkettige Fettsäuren und mikrobielle Biomasse – eine Vorstudie an Gras- und Maissilagen

*Zhang X., Hünerberg M., Hummel J. – Göttingen

Maximization of microbial growth is a major goal of any ruminant feeding strategy. Blümmel et al. (1) defined the partitioning factor ([PF]; ratio of truly degraded substrate and total gas production) as a reference for how much degraded substrate was incorporated into microbial cells or fermentation gases and short chain fatty acids, respectively. If relevant differences in PF exist among feeds, it would be interesting to identify potential impact factors, like cell wall content or the degradability. This study aimed to compare the PF of grass and corn silages and to investigate its relation to NDF, XP and XL content and NDF degradability.

Methods: Eleven grass silages (181 ± 30.7 g XP/kg DM, 41.8 ± 5.89 g XL/kg DM, 522 ± 34.6 g aNDForg/kg DM and 10.0 ± 0.74 MJ ME/kg DM) and ten corn silages (71.5 ± 7.88 g XP/kg DM, 28.6 ± 3.71 g XL/kg DM, 414 ± 32.6 g aNDForg/kg DM and 10.9 ± 0.35 MJ ME/kg DM) were tested using the Hohenheim Gas Test (HGT). About 200 mg DM of sample were incubated in gas-tight HGT syringes for 24 h. The gas volume was recorded and substrate residues were collected in Gerhardt fibre bags. The bags were boiled in neutral-detergent solution. The partitioning factor was calculated as ratio of truly degraded organic matter and total gas production. The data were analyzed using a linear mixed model approach in R (package "lme4") with the fixed effects of XP, XL, NDF and NDF degradability and the random effect of HGT run. A one-way ANOVA was performed to compare the means of PF of grass- and corn silages. Significance was declared at $P<0.05$.

Results: True organic matter degradability and 24-h gas production were 77.5 ± 6.4% and 44.3 ± 5.7 mL/200 mg DM for grass silage and 72.6 ± 2.5% and 53.5 ± 2.2 mL/200 mg DM for corn silage, respectively. The NDF degradability of grass silage and corn silage was 62.8 ± 8.4% and 36.9 ± 3.8%, respectively. The PF of grass silage (3.07 ± 0.27 mg/mL) was larger ($P<0.001$) compared to corn silage (2.63 ± 0.10 mg/mL). The NDF degradability of grass silages had a negative effect on PF ($P<0.001$), while XP had a positive effect on PF ($P<0.001$). The NDF and XL showed no effect ($P>0.1$). In corn silages, NDF degradability, XP and NDF had no effect on PF ($P>0.1$), while XL had a positive effect ($P=0.011$).

Conclusions: In this preliminary study, grass silages appeared to incorporate a higher proportion of degraded substrate into microbial synthesis than corn silages. Grass silages were characterized by a larger range of PF; a high protein content and surprisingly a low NDF degradability were associated with improved efficiency of microbial synthesis.

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* *Georg-August-Universität Göttingen Department of ruminant nutrition, Kellnerweg 6, 37077 Göttingen, Germany, zxy1019041587@gmail.com*

Workshop

Importance of livestock husbandry and nutrition in a circulatory system

Forage based production of milk and meat

Grobfutter als Basis der Erzeugung von Milch und Rindfleisch

Spiekers H., Ettle T. – Grub

Introduction

Because of the capability for ruminal fermentation, ruminants are also able to digest roughages which can hardly be utilized by monogastric animals. The most important forages are grass products from grassland or from arable fodder cropping as well as maize or grain whole plant silages. Moreover, clover or alfalfa products as well as their mixtures with grass are of relevance. The use of grass products from permanent grassland hardly causes any competitive situation to human nutrition. The higher the proportion of grass products in the ration is the lower is the nutrient import with bought-in feedstuffs. Moreover, in cattle there is a high evolutionary adaption to grass products. The following overview aims at illustrating which proportion of grass products in diets is fesible in respect of a more sustainable nutrition of cattle. In the meanwhile the importance of maize silage is comparable with that of grass products. This item will also be addressed.

Forage production and feed management

In Germany, about 29 % of the agricultural land are used as permanent grassland, about 14 % for maize silage production and about 4 % for production of other arable fodder cropping (grass, grass-clover mixture, alfalfa etc.) [1]. Based on harvested amounts the relations differ from mentioned data because of large differences in the crop yield [2]. Mean crop yield from permanent grass land in Bavaria is about 8 t dry matter (DM) per hectare (ha) and year and about 15 t DM/ha and year for whole plant maize. Arable fodder cropping allows yields of more than 10 t per ha and year. Large area covering information is not available, because a recording of the crop yield is not widely established [3]. Given that especially yields from grass land vary widely between different farms or fields [4], such a recording of yields would be highly desirable.

In respect to cattle nutrition the harvested amount is not of interest but the amount and the quality that can be utilized by the animal. Therefore different levels of yield are defined in the whole harvesting process with starting point at the field and its end at the feed bunk. In this context a very exact linguistic usage is necessary. Those different definitions of yield are described in the DLG-leaflet 416 [5].

The starting point for optimizing the use of forage for milk and meat production includes the reduction of the mass losses as well as the reduction of deterioration of fodder quality over the whole production process from the field to the bunker.

Figure 1 illustrates DM losses from grass and maize silage and the change in chemical composition of fresh forages compared to the ensiled material [6]. Despite the higher fermentability of maize in comparison to grass or clover products, the DM losses of different silages are comparable. This fact may be explained by losses due to reheating at the silo face. The reduction of those nutrient losses is a main factor of success in feedstuff management and animal nutrition. The respective measures for harvesting, feed preservation and fodder management at the silo and feed bunk have to be considered [7, 8]. Generally, a well justified application of silage additives is recommendable. In the foreground are the enhancement of lactic acid fermentation in grass products and the reduction of reheating in maize, respectively.

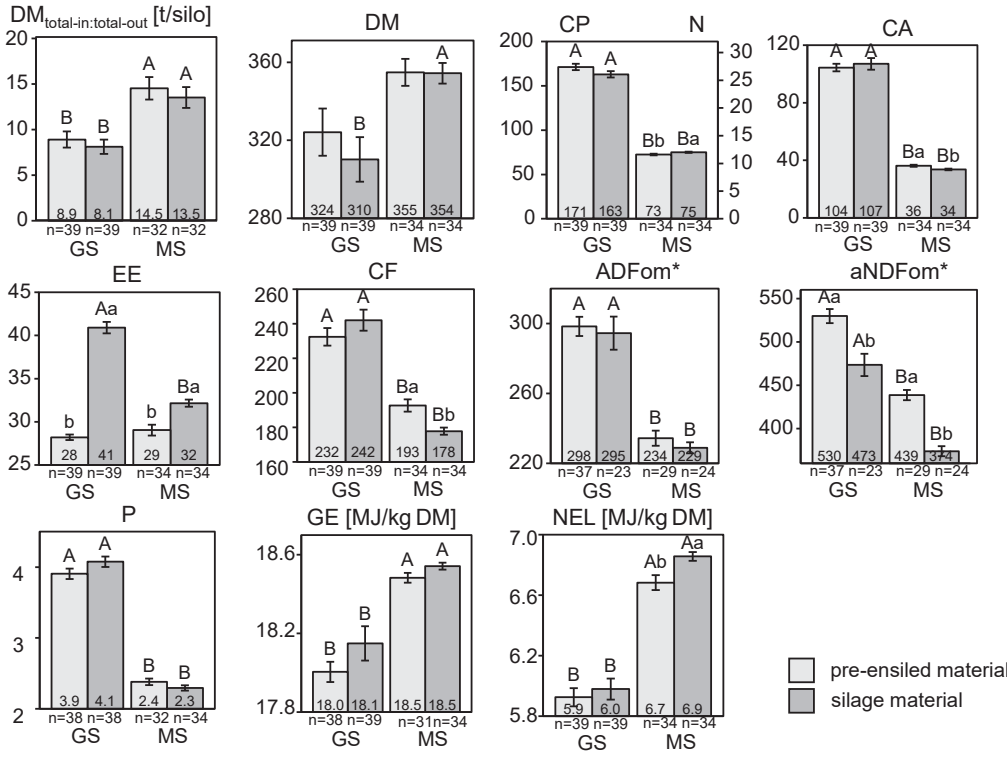

Figure 1 Comparison of means across groups of grass (GS) and whole-crop maize material (MS) regarding DM losses (DM(total-in:total-out)), nitrogen (N), phosphorus (P) and energy values (GE and NEL) (in g/kg DM unless stated).Various capitals = significance of crop (grass or whole-crop maize; $p < 0.05$), various lower case = significance of pre-ensiled or silage material ($p < 0.05$), n = number of analysis per silo; *Different numbers of ADFom/aNDFom analyses due to the lack of analyses from silages on one farm [6]

The increase of forage proportion in the rations results in higher quality demands of the used forages. Suitable benchmarks for nutrient and energy density in varying production categories are published [7, 9]. Also the quality of crude protein has a high importance. High proportions of rumen-undegradable protein as well as a low proteolysis are desirable. Therefore, silage production is in an inferior position compared to fresh forage, hay production or artificially drying.

Forage based production of milk and meat

The original use of grassland is grazing. However, characteristics of diets like feed composition, fiber content, the structural effectiveness of fiber affect feed intake. Even in very high quality pastures fiber concentration is high compared to total mixed rations (TMR) including concentrates. For this reason, dry matter intake and milk yield is restricted in grazing ruminants [10].

Grazing without offering additional feedstuffs can meet the requirements for maintenance and a milk yield of 25 kg/day when the concepts of good practice are applied. In heifers the requirements for maintenance and 800 g daily gain can be met. In suckler-cows of a breed with an intermediate milk yield the requirements of the cow and a calf having daily gains of about 1.200 g can be met. Thus, the energy and nutrient requirements of heifers and suckler cows can be met completely by grazing. However, the supplementation of minerals are generally necessary.

As potential for milk production is high especially in dairy breeds, a seasonal (block) calving system in the winter and pasturing without supplementation during grazing season is recommended [11]. In early lactation out of grazing season, concentrates supplementation allows covering the energy and nutrient requirements if cows are then barn fed. In the second and last third of lactation grazing without supplementation is sufficient to cover the cow`s energy and nutrient requirements, if the grown amount of grass is sufficient. Within different grazing systems the continuous intensive grazing system (short lawn pasturing) has been favored because this system is regarded to deliver a constant forage quality with an adequate structure for rumination.

The potential of such a system to produce milk from grass products is illustrated in table 1. Data are obtained from a 3-year feeding trial at the organically managed farm Kringell [12]. In order to evaluate the different systems, besides the milk yield per cow the milk yield per ha of land used for milk production is also displayed. Taking into account the amounts of concentrates supplied to the animals, the calculations also considered the respective land area based on measured yields for cereals and fodder legumes, respectively.
Obviously, the continuous grazing system has an increased output per unit land area under the agronomical conditions given at the farm Kringell. This is also true for the economic aspects. The obtainable milk yield (ECM, including maintenance requirement) from roughage was calculated to be 5,921 kg per cow and year in the continuous grazing system.

Table 1: Performance per cow and per ha land used in the comparison of the shed herd with year round calving and 2 ha pasture and the grazing herd with winter calving up to 14 ha pasture over 3 years (36 cows per herd) [12].

criteria	unit	shed herd	grazing herd	difference
ECM yield per cow	kg/a	8,833	7,555	1,278
concentrates intake	t/cow/a	2.37	0.72	1.65
ECM form roughage	kg/a	3,458	5,921	2,436
calculated ECM production per ha land [1)]	kg/a	8,048	8,924	876

1) including the land for the used concentrates; ECM – energy corrected milk with 3.4 % protein and 4 % fat

In solely barn fed cows the conditions for production of high milk yields from forage differ from grazing systems due to the mentioned mass and quality losses in the preservation process. The necessary amounts of concentrates in feeding high yielding dairy cows according to their requirements under consideration of environment and economy are presently discussed conversely. In the joint project „optiKuh“ (www.optiKuh.de) the effects of varying feeding intensities due to varying roughage qualities and concentrates levels on feed intake, milk yield, body condition, health and fertility were investigated. The feeding trials were conducted on a couple of research farms over a period of two years. Some results are shown in table 2 [13]. It can be basically concluded that usage of different concentrates levels is possible without any impairment of the measured criteria. A prerequisite is a sophisticated management according to the existing recommendations, especially for a good start into early lactation [14].

Table 2: Influence of roughage quality and concentrates level on feed intake and performance of German Holstein and Fleckvieh cows in the feeding trials from the joint project optiKuh [13].

MJ NEL in roughage, /kg DM	**6.1**		**6.5**	
cncentrates level, g/kg ECM	**150**	**250**	**150**	**250**
German Holstein (Body weight: 658 kg)				
feed intake, kg DM/day	20.6a	21.7ab	20.9a	22.7b
ECM, kg/day	28.5a	29.7a	30.4ab	32.0b
energy expenditure, MJ NEL/kg ECM	4.6	4.9	4.6	4.9
energy balance, MJ NEL/day	-0.5a	9.3bc	0.6ab	11.4b
Fleckvieh (Body weight: 750 kg)				
feed intake, kg DM/day	18.4a	20.4bc	19.5ab	20.6c
ECM, kg/day	25.7a	27.5ab	27.3ab	28.5b
energy expenditure, MJ NEL/kg ECM	4.6	5.0	4.8	5.0
energy balance, MJ NEL/day	-8.3a	2.2bc	-1.3b	4.9c

ECM – energy corrected milk with 3.4 % protein and 4 % fat

The amounts of roughages of 46 to 61 dt DM/cow and year fed in the feeding trials were relatively high compared to the common agricultural practice. Depending on concentrate proportion and quality of roughages the cows produ-

ced 4,000 to 7,000 kg milk per year solely from roughages [14]. Highest milk yields per kg of consumed roughages were observed when roughages of high quality were fed as TMR [15]. In the second half of lactation it was possible to reduce concentrates supply without any noteworthy effect on feed intake or milk yield, respectively. However, the cows were kept in a constant group throughout the trial. The individual access to the automatic feeding troughs guaranteed the establishment of the different TMR feeding groups. The results of the feeding trials revealed that a high energy concentration of roughage is the basis for high milk yields per cow of consumed roughage. The amounts of roughages which are displaced by an increased concentrates intake are lowest in early lactation, because energy requirement is higher than in late lactation. Therefore it is recommendable to use concentrates preferably in early lactation in order to obtain high milk yield based of roughage [16].

There is also some discussion on an absolute exclusion of concentrates from dairy cow feeding [17, 18]. However, based on the presented results such a feeding practice cannot be recommended under German feeding conditions. A promising approach to increase milk production from roughage is the production of high quality artificially dried grass products. If those local produced feedstuffs are fed, it is possible to reduce of concentrates and protein without impairment of animal performance [19]. However, artificially drying processes are often based on fossil fuels.

An additional purchase of concentrates leads to a considerable import of nitrogen and phosphorus into the materials cycle of a farm. The respective values based on the results from the feeding trial in Grub are presented in table 3 [20].

Table 3: impact of different amounts of concentrates on farm balance - optiKuh-trial Grub (Fleckvieh 4 x 24 cows, two years trial)

concentrates, dt/cow/a	13.1	22.6	13.4	20.9
ECM, kg/cow/a	7,194	8,235	7,673	8,396
nitrogen (kg/cow/a)				
in concentrates*	39	68	40	63
in milk	39	44	42	45
Balance	0	24	-2	18
phosphorus (kg/cow/a)				
in concentrates*	7.5	12.9	7.6	11.9
in milk	7.2	8.2	7.7	8.4
Balance	0.3	4.7	-0.1	3.5

* 30 g N and 5.7 g P per kg; ECM – energy corrected milk with 3.4 % protein and 4 % fat

It is also possible to produce considerable amounts of meat from grass, grass/clover or alfalfa products in cattle fattening systems [21]. However, the results of a feeding trial with Angus bulls from a suckler cow system suggest that the production of fattening bulls solely on the basis of grass products is not promising. Daily gains, carcass quality as well as economic traits were significantly improved when 1 or 2 kg of cereals per day were supplied in addition to grass silage offered for ad libitum intake [22].

Need for research

The optimum concentrate supplementation additionally to the roughage basis in ruminant nutrition is well investigated. They resulted in exactly defined recommendations for feeding practice. Some open questions remain regarding the feeding technique, e.g. organization of TMR feeding or the best practice in offering concentrates during lactation. For example it appears rather unclear whether a flat-rate feeding system (concentrates supply according to stage of lactation) provides advantages over concentrates supply according to animal individual milk yield. Moreover, open questions regarding protein degradation due to forage preservation and reduction of mass and quality losses from the field to feed bunk and the milk tank have to be clarified. The controlling of the feeding system also needs to be further investigated. Ecosystem services have to be highlighted if different systems are compared in future. This is true for the internal nutrient fluxes, the biodiversity as well as for the climate-damaging emissions. Furthermore, new concepts for the reduction of feed selection by the animal have to be taken into account. In this

context it has to be assessed in which manner those concepts concern to animal welfare aspects, if the restriction in the possibility for choice limits specific behavioral needs of the animals.

Conclusions

Forage based production of milk and meat can be managed very successfully. In this respect, the grass products compete with silage maize. Crop yields in production of silage maize are higher compared to yields of grass products, but operating costs are lower. In order to maximize production of milk or meat based of roughage, minimizing the losses from the field to the bunk is the major goal in further optimization of forage based production systems. Wherever possible, grass products should be fed fresh without preservation treatment. The appropriate use of concentrates allows to enhance efficiency of production systems especially in early lactation or in finishing period of fattening. The optimum levels depend on the prerequisites in the individual farm and the actual relation of costs for varying feedstuffs. The main objective is a more sustainable organization of feed production and feeding. The development and implementation of respective measurement criteria is necessary in order to be able to control the different production systems.

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* *Bayerische Landesanstalt für Landwirtschaft Institut für Tierernährung und Futterwirtschaft, Prof.-Dürrwaechter-Platz 3, 85586 Grub, Germany, hubert.spiekers@lfl.bayern.de*

Use of by-products in ruminants

Nutzung von Koppelprodukten beim Wiederkäuer

Gerlach K.[1], Hummel J.[2] – [1]Bonn / [2]Göttingen

Introduction

By-products of food and of biofuels have a long tradition of utilization in animal nutrition. The extensive use in this way can be considered an old (and sometimes underestimated) success-story of sustainability in animal production. An enriched fiber fraction represents a characteristic of many by-products. In consequence, ruminants appear particularly well-suited consumers, obviously due to their high fermentative and comminution capacities. According to their digestibility and nutritional quality for ruminants, by-products can be separated into high-quality (~ concentrate; e.g. beet-pulp, rapeseed meal, brewers spent grains, dried distiller grains with solubles (DDGS)), intermediate (e.g. wheat bran, apple pomace) or extremely low-quality feeds (straws; rice straw/bran). While a plethora of research has focused on aspects of by-product use in ruminants, the increased desire to minimize resource competition with human nutrition and to increase resource use efficiency has renewed the interest. The following aspects are among those keeping them a rewarding topic for investigations:

1) Considerable changes in the spectrum and quantity of available by-products have been seen over the last years, which can be expected to continue (e.g. by-products from biofuels).

2) Changes in nutritional quality can be expected in consequence of developments of production techniques.

3) Globally and regionally, efforts are increased to minimize environmental impact and resource competition of animal production, which encourages investigations on efficient use of established and new materials.

4) Efforts in the aforementioned fields may at some point also include the question of differences between animals in their use-efficiency of fiber-rich by-products.

Types and amounts of by-products

Using by-products as feedstuffs is a common practice worldwide but there is a lack of data documenting the recent amounts, especially on a global scale. Many by-product feedstuffs cannot be quantified because no standard equivalent is available in the literature to make this conversion from a processed food to a by-product feedstuff. (1) defines the by-product feedstuff equivalent as the percentage of an unprocessed commodity that becomes a by-product feedstuff on a dry matter (DM) basis.

For Germany, precise data are available on the amounts of by-products used as ingredient for compound feed production (2). In 2017/18, about 24 Mio. tons (t) of compound feed were produced in Germany with grains being the most important ingredients (11.6 Mio. t). By-products from the oil industry (cakes/expeller/meals) were highly used with 6.1 Mio. t, followed by brans (by-products of milling grains) with 1.7 Mio. t, maize gluten meal/feed with 470.000 t, DDGS (270.000 t) and sugar beet pulp (by-product of sugar production from sugar beets) with 238.000 t. Citrus pulp and pomace, once being very popular, is nowadays much less used than years before (15.100 t compared to 556.000 t in 1995/96). Besides the use as compound feed ingredients, there are large quantities of by-products which are fed directly on farm, especially in ruminant nutrition. In this regard, brewers spent grains, sugar beet pulp, DDGS and straw provide the largest amounts but not in every case, precise data are available. In 2017/2018, a total amount of 3.1 Mio. t of beet pulp (fresh weight, different types, wet and dried) was produced, equaling approximately 1.6 Mio. t DM (3). Comparing those data with the amounts of beet pulp used as compound feed ingredient (238.000 t) it shows exemplarily the importance of the direct on-farm use for by-products in ruminant nutrition. During the last years, several shifts have occurred in the share and total amounts of by-products used in ruminant nutrition. The use of DDGS in compound feed production, for example, increased within five years from 82.000 t in 2012/13 (first time listed) to 270.000 t in 2017/2018 (2). Also the use of by-products from starch industry (maize gluten feed and meal, potato pulp) is expected to rise as increasing amounts of recycled paper are produced (e.g. for parcel delivery) which requires the use of starch as a filling material (~16.2 kg starch/kg paper) (4).

Many of the aforementioned by-products that are fed directly on farm are relatively humid (DM 20-30%) and therefore prone to spoilage, meaning that they have to be fed within a few days or conservation techniques (e.g. ensiling) have to be applied. Furthermore, by-products often contain elevated concentrations of P (compared to the initial substrate) that need to be considered in ration formulation. Especially imported products repeatedly attracted attention due to increased concentrations of contaminants like aflatoxins or dioxins (e.g. in citrus pulp) which can lead to shifts in demand.

On a global scale, precise amounts of by-products used in animal nutrition are more difficult to quantify. For the

year 1993, crop residues from wheat and rice, soybean and rapeseed meal and cakes, maize gluten feed and meal as well as bagasse (from sugar cane) and brans were mentioned as the by-products with the highest quantity worldwide (1). It is estimated that for every 100 kg of food and fiber consumed or utilized by humans, at least 37 kg of by-product feedstuffs are generated that can be consumed by livestock (1). With a further growing human population also the amount of by-products available as feedstuff increases. Based on production data provided by FAO for single agricultural products it is possible to make a rough estimate for the quantity of by-products arising from that product. This is exemplarily done for almond hulls, which are the by-product occurring during harvesting and processing of almond nuts. Currently, almond hulls are mostly used as cattle feed and shells as bedding material (5). The proportion of the hull, shell and nut is 50:25:25 (air-dry basis). In 2017, ~2.24 Mio. t of whole almonds were harvested worldwide, meaning that 1.12 Mio. t of almond hulls were available (FAO, 2019). During processing, two further by-products, in addition to the shell and the hull, are produced (blanched skin, representing 4.0–8.0% of the total shelled almond fresh weight, and blanch water), such that 70.0–85.0% of the whole almond fruits constitute residues of this agro-food activity at the end of processing (5).

Brewers spent grains as a by-product from beer production is popular as ruminant feedstuff nearly all over the world. From the production of barley beer (6), approximately 4.9 Mio. t DM of brewers grains were produced worldwide in 2014. The extraction of starch from barley/malt during the brewing process results in a doubling of the crude protein concentration which makes it a valuable protein source for ruminants.

As mentioned before, crop residues from wheat and rice production provide huge quantities of (low quality) by-products. In 2017, 770 Mio. t of rice were produced worldwide (6). Calculations of crop residues from grain production assume that crop residues were 55% and grain was 45% of the total mass. In addition, only 50% of the residue generated can be considered available as a by-product feedstuff because 50% of the crop residue is left on the field for agronomic reasons (1) such that approximately 470 Mio t of rice straw should be available worldwide. For South East Asia (including China and Mongolia) the calculated utilization of rice straw for animal feeding was 30-40%, reflecting considerable underutilization and hence major opportunities for promoting more intensive use (7). Improvements in its nutritive value e.g. by pre-treatments (see below) could have an immense impact on animal production in those countries that often lack roughages and adequate feed during dry season.

Another interesting source of by-products for ruminants is the fruit and vegetable production and processing industry. With the growing human population and changing diet habits, the production and processing of horticultural crops, especially fruits and vegetables, have increased very significantly to fulfill the growing demands (8). The processing operations produce significant amounts of by-products that can constitute about 25% to 30% of a whole commodity group, plus products not used during harvest, storage and consumption. For example, fruit and vegetable processing, packing, distribution and consumption in the organized sector in India, the Philippines, China and the United States of America generate a total of approximately 55 Mio t (1.81, 6.53, 32.0 and 15.0 Mio. t, respectively) of wastes and by-products per year which are mainly composed of seed, skin, rind and pomace and a large proportion of these wastes are currently dumped in landfills or rivers, causing environmental hazards (9). Although being available in huge quantities, the use of byproducts from the processing of fruits and vegetables has not been implemented on a large scale. For a continuous use as feedstuff, most of those often very humid products need to be conserved, e.g. dried or ensiled (co-ensiling with drier substrates).

Particular characteristics of by-products

Besides the benefit of being particularly sustainable feeds, many by-products are advantageous simply due to their attractive nutritional characteristics. By-products rich in easily fermentable fiber like beet pulp represent a classic example. It contains high amounts of easily fermentable cell wall components like pectin and non-lignified hemicelluloses and cellulose. Characterized by high ruminal digestibility and very high fermentation rate, it is known for its physiologically favourable fermentation pattern (which means that its effect on ruminal pH is less than what could be expected from its energy content). Different explanations for this observation have been proposed, like its fermentation pattern yielding a lot of acetate (in contrast to increased propionate and potentially lactate from starch-rich concentrates), the pH sensitivity of the microbes responsible for the degradation of cell wall carbohydrates (with some potential for self-regulation of fermentation) or a relatively high intrinsic pH buffering capacity (10). Beneficial effects on milk yields/avoidance of excessive body condition at late lactation stages have also been postulated for by-products rich in soluble fiber (11).

Further by-products belonging to this group are citrus pulp (having largely disappeared from the German market, as mentioned above), carrot pomace (with some importance e.g. for organic agriculture) or soybean hulls. When comparing fermentation characteristics, considerable differences become obvious, related to carbohydrate composition: E.g., soy bean hulls with a high proportion of non-lignified cellulose are characterized by a high degradability but a low fractional fermentation rate [4 %/h] (12), which is in contrast to the very high fractional fermentation rate of pectin as a dominant fiber component of beet pulp (~ 20 – 40%/h).
Another area of by-products with particularly attractive characteristics for ruminants are protein-rich residues with a high proportion of rumen-undegradable crude protein (RUP, as proportion of total crude protein) like rapeseed meal (solvent-extracted) (35% RUP), brewers spent grains (45% RUP) or DDGS (40% RUP).

Innovations with potential to broaden use of by-products

For by-products characterized by low digestibility, efficient use is restricted mostly due to lignification. Different treatments have been investigated to increase the value of such feeds, largely straws (13). Approaches reach from physical treatments to the addition of chemicals (NaOH; ammonia or urea) or biological treatments (rot fungi). While the former two approaches appear less promising nowadays due to economic and environmental reasons, the latter, despite long known and investigated (14), should still be considered a promising approach to substantially improve efficiency of low quality by-products (15) on a broad scale. Direct application of enzymes can be regarded a further promising approach today (16). It is clear that any biological approach (applying organisms or enzymes) will only be effective under aerobic circumstances, since the presence of oxygen is mandatory for lignin degradation. This limitation is also true for the lignin degradation activity seen in some insect digestive tracts, which appears to be restricted to the aerobic sections (17). While lignification is the major challenge in most straws, high silica proportions contribute to the low quality of feeds like rice straw (18). Being known for increasing tooth abrasiveness, a depressing effect on digestibility is a major effect of silica in feeds (19).

Use of by-products by ruminants – comparative aspects

While among the diversity of large mammalian herbivores, a negative correlation of fiber content and DM intake is a general pattern (20), some superior capacity of monogastrics (like equids or elephants) to process highly fibrous feed is well documented. Among domesticated ruminants, some differences in their potential to process fibrous materials can be assumed. Goats are often described to have a particular capacity to process and digest fiber-rich, highly lignified feeds; however, it is not clear to what extent this is due to their preference for browse material, which may at least partly be due to their particular tolerance of plant deterrents. In general, body mass (BM) could be expected to be correlated positively with fiber tolerance, since rumen (and whole gastrointestinal tract) volume scales to BM1.0 (21), while energy requirements do so to BM0.75, which results in an advantageous ratio for larger BM. However, while this relation can be calculated for interspecies comparisons, it does not appear to be present when comparing breeds, since extensive breeds often appear to be characterized by a relatively small body mass. Among the characteristics relevant to qualify a ruminant as a successful fiber processor can be expected a) food comminution (e.g. teeth structure and surface), b) rumen volume/gut capacity, which has been shown to be more adaptable in extensive breeds (22), c) passage rates, d) capacity of ruminal particle retention mechanism to pass on indigestible particles, e) urea recycling capacity (allowing diets lower in N) or f) a particular microbiome.

Conclusion

In the food production chain, ruminants play an important role as efficient utilizers of fiber rich feeds, including considerable amounts of by-products. For many of them, inclusion in ruminant diets is already maximized due to their high nutritional value. For others, mostly those high in lignified fiber, innovations to allow maximization of their incorporation in ruminant diets would be highly welcome, but need further investigations and optimizations. Successful approaches are likely to differ on a global scale.

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* *University of Bonn, Faculty of Agricultural Sciences, Institute of Animal Science, Endenicher Allee 15, 53115 Bonn, Germany, kger@uni-bonn.de; University of Göttingen, Faculty of Agricultural Sciences, Department of Animal Sciences, Ruminant Nutrition, Department für Nutztierwissenschaften, Kellnerweg 6, 37077 Göttingen, jhummel@gwdg.de*

Industrial by/side-products as feed stuffs for pigs and poultry. Bonus or burden?

Industrielle Nebenprodukte als Futtermittel für Schwein und Geflügel. Fluch oder Segen?

Schedle K.* – Vienna

In the context of the steady reduction of natural resources due to an increasing human population, one major challenge for a sustainable animal production is the improvement of its efficiency and the application of feedstuffs which are not in competition to human food (1).

Furthermore, the request for reducing the dependency on imports as well as for GMO-free feeding strategies have encouraged numerous experiments to increase the degree of self-supply using alternatives to soybean meal. In addition to legumes, especially the side/by-products of oilseed processing and of the bio-ethanol production rank among the traditional dietary protein sources. Possible limitations for the use of feed stuffs of plant origin are their secondary plant constituents, some of which showing anti-nutritional properties. However, these substances could be reduced significantly due to achievements in plant breeding and feed technology. This permits higher inclusion levels of alternative protein sources or feed stuffs which are not in competition to human food (2). Up to now, a broad range of methods like feed additives or technological treatments has been available to improve the nutrient efficiency of industrial by/side-products and hence the production of pig- and poultry-derived food. Nevertheless, the exact knowledge of the mode of action of these tools is a prerequisite for their successful application. Furthermore, information concerning their impact on the nutrient availability of the different feedstuffs is of great importance, in order to formulate diets that cover the animals‘ requirements and to avoid wasting valuable nutrients (1). This abstract is intended to provide an overview of the possibilities of selected industrial by/side-products, applied as feed stuffs for pigs and poultry.

The interest in producing ethanol from cereals for substitution of motor fuels is increasing worldwide. As a result, the process of cereal based ethanol production yields side/by-product, dried distillers grains with solubles (DDGS). DDGS contains large quantities of nutrients including energy, crude protein (CP) and non-phytate phosphorus. Hence, it has the potential for being used as feed to monogastric livestock like pig and poultry.

Our studies indicate that grower-finisher pigs tolerate DDGS as sole protein source (3,4). Broiler chickens accept DDGS contents up to 24% in their diets, without impairing important zootechnical and carcass parameters (5). A precondition, however, is a sufficient supply with essential nutrients to cover the animal’s requirements (3,4,5). The increasing dietary fibre or non starch polysaccharides (NSP) and CP content induced by DDGS, does not alter protein fermentation but carbohydrate fermentation in the intestine. Supplementation of NSP-degrading enzymes showed no impact on zootechnical performance and carcass parameters in diets containing high amounts of DDGS. Hence, the applied NSP-hydrolysing enzymes do not yield an advantage in combination with the DDGS product (4,5). The inclusion of similar NSP-degrading enzymes in the production process of the applied DDGS product may be an explanation for the absence of distinct effects of the NSP-hydrolysing enzyme supplemented in the diet on performance in high DDGS treatment. Regarding performance parameters, similar results were observed for 00 varieties of rapeseed meal in fattening pigs (6) or laying hens (7).

Wheat bran is a side-product of the milling industry and its annual production volume accounts about 150 million tons. Wheat bran contains relatively high amounts of dietary fibre or NSP. Hence, the digestibility is hampered, and this limits its application in animal feeding. However, as a feed stuff which is not in competition to human nutrition, it could be important for a sustainable production of food of animal origin, in the special case of an improved nutrient availability. Despite its nutritional value related to ingredients like starch and minerals, its high amount of dietary fibre possesses physiological and technological drawbacks, such as low digestibility and detrimental sensory properties (8,9). Moreover, feed stuff rich in phytic acid like wheat bran can interact with several minerals and proteins and subsequently inhibit the absorption of these compounds (10).

Our studies indicate that piglets and laying hens tolerate wheat bran up to 15% in their diets, without impairing important zootechnical, carcass, and egg quality parameters, respectively (8,9,11). Again, for a successful application of such a feed stuff in diets for pigs or poultry, a sufficient supply with essential nutrients to cover the animal’s requirements is necessary (8,9,11). Furthermore, our studies show that microbiota composition, as well as intestinal physiology seems to be stable and strongly depend on the gut segment than on the diet composition (8,11).

Feed treatments based on a hydro-thermal technology like pelleting, expanding or extruding are well established in the feed industry. Feed processing further adds to the cost of feeds. However, it provides an opportunity to improve the animals’ performance (1,12).

Among the methodologies used for the modification of feed materials, hydro-thermal technologies are known to exert a major influence on nutrient absorbance in the gut, but this effect strongly depends on the botanical source (13). In conventionally ground, dietary fat-rich feedstuffs like cakes, a substantial amount of oil may be encapsulated in the cell wall and hence in the dietary fibre/NSP fraction (12). Moreover, the nutrient-encapsulating effect of dietary fibre/NSP in fibre-rich industrial side/by-products is well known (14). New literature reports that the improved digestibility and metabolizable energy content (up to 17.5%) of industrial side/by-products, which are rich in fibre and low in starch, is a result of hydro-thermal or fermentative treatment (12). As a result, it is possible to obtain a higher inclusion level of wheat bran in diets for pigs and poultry. Interestingly, these observations were not so pronounced in piglets (11). A possible reason for this phenomenon could be the manner in which the young pigs' digestive system adapts to fibre-rich diets (15). However, high temperatures may induce other changes in the feed that may negatively affect its nutritional value. One negative effect may be the formation of complexes between fat and starch in the feed (1). Other negative effects include reduced protein digestibility and the loss of heat-labile components, such as vitamins and enzymes (16).

In addition to hydro-thermal treatments, further techniques like fermentation, germination or soaking show great potential to improve nutrient utilisation in pigs and poultry (17). In recent years, fermentation of dry feeds was effectively used to improve nutrient digestibility in diets for non-ruminants (17). During fermentation process, nutrients become more usable for the animal (10,12,17).

In summary, feed treatments in the form of a fermentation process or a hydro-thermal application can be a useful tool for improving nutrient availability and hence performance. However, in the case of wheat bran, fermentation seems to be more useful than a hydro-thermal treatment (12).

Conclusion and perspectives

Above all, pigs and poultry directly compete with humans concerning feed-/foodstuffs, due to their similar digestive physiology. Hence, the inclusion of industrial by/side-products in high amounts in pig and poultry diets provides a significant contribution to solving the problem between feed and food. Currently, there is a combination of different tools that offers a promising way of improving sustainability in pig and poultry production systems. As a result, the consumption of natural resources like grains can be considerably reduced and the substitution of regional industrial side/by-products like wheat bran, dried distillers grains with solubles or rapeseed meal can be dramatically enhanced in diets for pigs or poultry, thus contributing to more sustainable livestock production. Hence, by improving the efficiency of the production of animal-derived food, the term ‚'sustainable'' remains highly significant. Increasing efficiency plays an important role in ensuring that the resources required for pig and poultry nutrition are foreseeably available.

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* *Institute of Animal Nutrition, Livestock Products, and Nutrition Physiology (TTE), Muthgasse 11, 1190 Vienna; EMail: karl.schedle@boku.ac.at*

Reserves and solutions for livestock feeding from plant breeding

Reserven und Lösungen für die Nutztierfütterung aus der Pflanzenzüchtung

Doleschel P., Mohler V. – Freising

Background

Population growth and climate change are continuing and there are increasing concerns regarding the basics of human nutrition including the competition between farm animals and humans (1). Future livestock feeding should not only take into account residues from the processing of food and biomass or the use of crop residues, but also optimized grassland use.

Potential use of plant residues and by-products for livestock feeding

Table 1 shows recent acreage and production of major crops (2) and their by-products that can be used for feeding. Residues that are not immediately usable for human nutrition are crop residues, i.e. straw, residual plants such as beet leaf or potato herb and by-products from industrial processing including bran, stillage, gluten, spent grains, dried distillers grains with solubles (DDGS), molasses, and beet pulp. Residues from oil production (extraction meal, cakes) are of particular importance as they can also be used for human nutrition (soybean meal).

Insert table 1 here!

Reserves for an increased use of plant residues as animal feed can be seen in the crop residues - especially straw. Besides a limited suitability as feed, competitive relationships also play a role: An energetic use of straw is an alternative. Feeding or energetic use both compete with build-up of soil organic matter.

Beet leaves offer a potential that is currently largely untapped. A re-entry of this substrate into use as animal feed is likely to be difficult because of technical and organizational challenges and the not always unproblematic preservation.

Plant breeding for improved feed quality

To expand the use of plant residues and by-products or to make previously unsuitable substrates available, improvements in limiting properties such as digestibility, content of anti-nutritional components or content of essential amino acids are desirable. This is where plant breeding will make a significant contribution.

In addition, the sustainable use of grassland should also be considered. Targeting robust plant communities is necessary. Resistance to drought stress and heat can be improved by re-seeding or over-seeding with appropriate seed mixtures. Here, improved varieties are available or under development.

Table 2 shows general breeding goals as well as those specifically geared towards feed use.

Insert table 2 here!

The development of plants with new or improved properties is a time and cost intensive process. The improvement of quality traits requires additional effort for the „phenotyping“ of ingredients. Here, molecular marker-based approaches (5) developed over the last 30 years offer the opportunity to make faster progress.

For sustainable practical approaches - beyond publicly funded projects - plant breeding needs impulses from the market. This essentially includes rewarding improved properties on the market so that specially adapted varieties experience sufficient demand.

There is no doubt that the new breeding technologies (NBT) offer new perspectives (8). Especially for a more environmentally friendly production of feed through higher N efficiency or reduced N2O release, there are promising approaches using NBT (9, 10), although some of these cannot be regulated in the EU at present.

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Table 1: Important crops and possible use of residues for feeding

Crop	Area harvested 1.000 ha	Production t/ha	By-product	Amount by-product 1.000 t	Usage options for residues and by-products
Cereals	6.276	42.641	straw, bran, DDGS	34.000 (straw, est.)	Use of straw, bran and spent grains is well established
Maize (grains or corn cob mix)	423	3.813	Straw, Corn gluten feed	1.500 (straw, est.)	Maize straw can be used as biogas substrate; use for feed is conceivable but not expedient
Grain legumes	195	515	Straw	250 (straw, est.)	Use of straw for feed is conceivable but the straw is often not dry enough
Potatoes	260	10.353	Mash, potato protein	-	Industrial by-products are established as feed; Use of leaves/foilage makes technically no sense
Sugar beet	411	28.783	Leaf	10.000 (leaf, est.)	Beet leaf is hardly used as animal feed; Use of by-products of processing is established
Oilseed rape	1.129	3.593	Rape seed meal, straw	2.000 (meal, est.) 1.600 (straw, est.)	Rapeseed meal and rapeseed cake are established as animal feed; Use of straw is conceivable but makes technically no sense
Silage maize	2.172	87.443	-	-	-

Data Source: (2, 3), average 2017-2019, own calculations and estimates (est.)

Table 2: Breeding goals and use of modern breeding techniques in relevant crops (4, 5, 6, 7, 8)

Crop	Breeding goals	Reported use of modern breeding techniques	Feed value relevance
Wheat	Yield, yield structure, early maturity, winter hardiness, disease and pest resistance, protein content, milling and baking quality, pre-harvest sprouting resistance, drought tolerance	Marker assisted selection (MAS): Resistance genes, Rht genes (plant height); Genomic selection (GS): Yield, protein content, resistance to pre-harvest sprouting, etc.; New breeding technologies (NBT): Gluten content	Amount of protein (low!) desirable: low P content (P balance; especially: bran) not considered: straw quality
Maize	Grain yield, dry matter yield and digestibility, digestible crude fiber, early ripeness, root strength and stem solidness, cold tolerance, disease and pest resistance, hybrid vigour, protein and oil content	MAS: Many traits; GS: Yield, energy density, digestibility, etc.; Gene transfer (GT): Many traits (e.g. herbicide resistance, insect resistance, stress tolerance) NBT: Disease tolerance, starch quality, phytate content etc.;	Protein quality, oil content, digestibility, Fusarium resistance
Oilseed rape	Seed yield, disease and pest resistance, oil content and quality, habitual resistance, winter hardiness, quality of the rapeseed meal	MAS: many traits; GT: Various traits, e.g. herbicide tolerance; NBT: Fatty acid composition, herbicide tolerance;	Low glucosinolate content of the rapeseed meal
Grasses: Lolium, Festuca, Festulolium, (Brachiaria)	Dry matter yield, competitiveness, winter hardiness, digestibility, protein content, sugar content, disease resistance, drought resistance, time of ripening (late varieties desirable)	MAS: Disease resistance (rusts) and special traits;	Digestibility, protein content, sugar content
Alfalfa	Dry matter yield, disease and pest resistance, protein content, seed yield (flower fertility and attractiveness), drought resistance (rapid youth development), nitrogen fixation, carotene content, durability at higher cutting frequency	MAS for some traits; GT: herbicide tolerance; NBT: Lower lignin content;	Desirable: Suitability for grazing (not very promising).
White clover	Dry matter yield, disease resistance, low cyanogenic potential, seed yield, nitrogen fixation	-	Desirable: Low content of anti-nutritional ingredients

* *Bayerische Landesanstalt für Landwirtschaft Institut für Pflanzenbau und Pflanzenzüchtung, Am Gereuth 8, 85354 Freising, Germany*

Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology

Mitteilungen des Ausschusses für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie

Gleichungen zur Schätzung der Umsetzbaren Energie und der Verdaulichkeit der Organischen Masse von Maisprodukten für Wiederkäuer

1. Einleitung

Für eine bedarfsgerechte Energieversorgung landwirtschaftlicher Nutztiere muss der energetische Wert der Futtermittel bekannt sein. Die Umsetzbare Energie (metabolizable energy, ME) ist der Maßstab zur Energiebewertung beim Wiederkäuer (GfE, 2001). Die Berechnung des ME-Gehaltes eines Futtermittels erfolgt aus den verdaulichen Rohnährstoffen gemäß folgender Gleichung (GfE, 2001):

[1]	ME (MJ/kg)	=	0,0312	• verdauliches Rohfett (g/kg)
		+	0,0136	• verdauliche Rohfaser (g/kg)
		+	0,0147	• verdaulicher organischer Rest (verdauliche Organische Masse – verdauliches Rohfett – verdauliche Rohfaser) (g/kg)
		+	0,00234	• Rohprotein (g/kg)

Bei der Arbeitsroutine in den Untersuchungslaboren werden für die Berechnung der ME Schätzgleichungen genutzt. Für Maisprodukte wurde von der GfE (2008) die folgende Gleichung veröffentlicht:

[2]	ME (MJ/kg TM)	=	7,15		
		+	0,00580	• ELOS	(g/kg TM)
		–	0,00283	• aNDFom	(g/kg TM)
		+	0,03522	• XL	(g/kg TM)

Eine Validierung der Gleichung 2 auf Basis von 120 neuen Verdaulichkeitsmessungen mit Maisprodukten aus den Jahren 2007 bis 2018 aus Deutschland, Österreich und der Schweiz ergab zwar einen mit 3,2 % geringen Schätzfehler, das Bestimmtheitsmaß war mit 0,58 jedoch unbefriedigend gering. Die Differenz zwischen den geschätzten und den aus der Verdaulichkeitsmessung ermittelten ME-Gehalten betrug bis zu 1 MJ/kg TM. Es wurde daher eine neue Schätzgleichung abgeleitet, die der Ausschuss für Bedarfsnormen hiermit für die zukünftige Anwendung für Maisprodukte festlegt. Zusätzlich wurde mit dem Datenmaterial eine Gleichung zur Schätzung der Verdaulichkeit der Organischen Masse abgeleitet.

Bereits in der früheren Mitteilung wurde die im Hohenheimer Futterwerttest gemessene Gasbildung als in vitro-Kriterium bei der Ableitung der Schätzgleichung für Maisprodukte nicht empfohlen (GfE, 2008). Auch die neue Auswertung führte dazu, dass die Gasbildung unberücksichtigt bleibt.

2. Daten und Vorgehensweise

Für die Auswertung wurden 156 Datensätze aus Verdaulichkeitsversuchen verwendet, die in neun Versuchseinrichtungen durchgeführt wurden (Tab. 1). Die Verdaulichkeit der Rohnährstoffe im frisch geernteten Silomais (n=10), in Maissilagen (n=141) und im silierten Restpflanzenmaterial (n=5) wurde nach den Richtlinien der GfE (1991) unter Nutzung von Hammeln im Zeitraum von 2000 bis 2018 bestimmt. Bei den silierten Materialien wurden die Trockenmassegehalte und die Nährstoffgehalte auf die Verluste flüchtiger Substanzen während der Trocknung nach den Vorgaben von Weißbach und Kuhla (1995) korrigiert.

Verwendete Abkürzungen: ADFom = Säure-Detergenzien-Faser nach Veraschung; aNDFom = Neutral-Detergenzien-Faser nach Amylasebehandlung und Veraschung; B = Bestimmtheitsmaß; ELOS = Enzymlösliche Organische Substanz; GE = Bruttoenergie; ME = Umsetzbare Energie; NEL = Nettoenergie-Laktation; NfE = Stickstofffreie Extraktstoffe; OM = Organische Masse; sR = Reststreuung; VQ OM = Verdaulichkeit der Organischen Masse; TM = Trockenmasse; XA = Rohasche; XF = Rohfaser; XP = Rohprotein; XL = Rohfett

Tabelle 1: Anzahl und Herkunft der Datensätze

Posieux	12
Braunschweig	11
Dummerstorf	16
Grub	17
Gumpenstein	36
Halle	2
Paulinenaue	20
Kleve	31
Freising-Weihenstephan	11

In der Tabelle 2 sind die Daten zur näheren Charakterisierung des Materials zusammengefasst.

Tabelle 2: Inhaltsstoffe, in vitro-Kriterien, Verdaulichkeit der Organischen Masse und berechneter ME-Gehalt für das Gesamtmaterial (Mittelwert, Standardabweichung s, Variationskoeffizient s%, Minimum und Maximum)

		n	Mittelwert	s	s%	Min	Max
Trockenmasse	g/kg	156	337	55	11,5	210	569
Rohasche	g/kg TM	156	39	8,3	21,3	22	75
Rohprotein	g/kg TM	156	75	8,7	11,5	52	106
Rohfett	g/kg TM	156	29	4,9	16,9	6	106
Stärke	g/kg TM	94	331	61,0	18,4	169	499
Rohfaser	g/kg TM	156	204	36,0	17,4	141	334
aNDFom	g/kg TM	156	421	64,3	15,2	271	690
ADFom	g/kg TM	156	230	42,3	18,4	145	437
Gasbildung	mL/200 mg TM	120	55,0	4,85	8,8	46,2	68,4
ELOS	g/kg TM	156	688	67,3	9,8	325	827
VQ OM	%	156	74,5	4,2	5,6	55,7	82,3
ME	MJ/kg TM	156	10,9	0,70	6,4	4,5	12,1

Das Datenmaterial wies mit Variationskoeffizienten zwischen 15 und 18 % für die Faserfraktionen, 11,5 % für Rohprotein, 16,9 % für Rohfett sowie von etwa 10 % für die ELOS eine ausreichend hohe Streuung zur Ableitung von Regressionsgleichungen auf. Für die Größen VQ OM und ME wurden mit 5,6 bzw. 6,4 % geringere Variationskoeffizienten ausgewiesen. Die Rohfasergehalte wurden im Rahmen der Charakterisierung des Materials ebenfalls ermittelt und sie werden zur Berechnung der Referenzwerte für die ME benötigt. Bei der Ableitung der Schätzgleichungen wurde die Rohfaser jedoch nicht als Variable berücksichtigt. Die Ableitung der Schätzgleichungen erfolgte auf der Basis der in den Verdaulichkeitsmessungen nach Gleichung [1] berechneten ME-Gehalte sowie der jeweiligen Nährstoffgehalte und der enzymlöslichen Organischen Substanz (ELOS) sowie der Gasbildung in der Trockenmasse. Für die Ableitung der Schätzgleichung für die VQ OM wurden die Nährstoffgehalte und die in vitro-Größen auf die Gehalte in der Organischen Masse berechnet.

Für die mathematische Ableitung der Schätzgleichungen wurden alle 156 Datensätze verwendet. In einem ersten Schritt wurden verschiedene Schätzgleichungen zur Vorhersage des Gehaltes an ME abgeleitet und anhand ihres Bestimmtheitsmaßes (B), der Reststreuung (sR) und des Bias miteinander verglichen. Die Gleichungen wurden mittels des Statistik-Datenpaketes SAS® (Version 9.4) unter Nutzung der Prozedur PROC REG bei schrittweiser Parameterauswahl ermittelt. Es wurden nur die Variablen berücksichtigt, die sich bei einem Signifikanzniveau von $p < 0,15$ als signifikant erwiesen.

3. Ergebnisse

3.1 Regressionsgleichung zur Berechnung der ME

Insgesamt wurden 35 Schätzgleichungen abgeleitet, deren Bestimmtheitsmaße zwischen 0,20 und 0,78 variierten. Die Einbeziehung der aNDFom erwies sich in keinem Falle als sinnvoll, da das Signifikanzniveau des Regressionskoeffizienten jeweils deutlich geringer als das der ADFom war. Bezüglich der in vitro-Größen zeigte sich ein deutlich engerer Zusammenhang zwischen dem ELOS-Wert und dem ME-Gehalt als zwischen der Gasbildung und dem ME-Gehalt, was sich mit früheren Befunden deckt (GfE, 2008). Die folgende Gleichung wird zur Schätzung der ME in Maisprodukten unter Berücksichtigung der Schätzgüte und des erforderlichen Analyseaufwandes festgelegt:

[3]	ME (MJ/kg TM)	=	9,46		
		+	0,00336	• ELOS	(g/kg TM)
		–	0,00636	• ADFom	(g/kg TM)
		+	0,01829	• XL	(g/kg TM)
		+	0,00865	• XP	(g/kg TM)
		–	0,01474	• XA	(g/kg TM)

Bestimmtheitsmaß r2: 78 %; Reststreuung sR: 0,34; Schätzfehler sR*100/ $\bar{x}$: 3,08 %

Für Futtermittel für Milchkühe kann die Nettoenergie-Laktation (NEL) aus der ME unter Berücksichtigung der Umsetzbarkeit der Bruttoenergie (GE) (q) gemäß GfE (2001) wie folgt errechnet werden:

[4] NEL (MJ) = 0,6 • [1 + 0,004 (q – 57)] ME (MJ), wobei q = ME/GE • 100

Dafür muss der Gehalt an GE, falls dieser nicht bombenkalorimetrisch gemessen wurde, zunächst mit der folgenden Gleichung berechnet werden:

[5]	GE (MJ/kg TM)	=	0,0239	• XP	(g/kg TM)
		+	0,0398	• XL	(g/kg TM)
		+	0,0201	• XF	(g/kg TM)
		+	0,0175	• NfE	(g/kg TM)

Die Validierung der Gleichung 3 erfolgte an einem unabhängigen Datensatz der bereits genannten Prüfeinrichtungen von 52 Verdaulichkeitsmessungen für Maisprodukte aus den Jahren 2000 bis 2018. Die mittlere Differenz zwischen den geschätzten und den aus der Verdaulichkeitsmessung ermittelten ME-Gehalten betrug 0,01 MJ/kg TM bei einem Bestimmtheitsmaß von 75 % und einem Schätzfehler von 3,6 %, worin eine ausreichende Genauigkeit gesehen wird.

3.2 Regressionsgleichung zur Schätzung der VQ OM

Es wurden auch Gleichungen zur Schätzung der VQ OM abgeleitet, da die Verdaulichkeit eine Voraussetzung für die Berechnung der Energiewerte ist, sie aber unabhängig von der Ausgestaltung eines Energiebewertungssystems bleibt. Zur Ableitung der Schätzgleichungen für die VQ OM wurden ebenfalls die Konzentrationen an Rohprotein, Rohfett, ADFom, aNDFom sowie mindestens ein in vitro-Kriterium verwendet. Die Berechnungen erfolgten auf der Basis der Gehalte in der Organischen Masse. Variablen, deren Regressionskoeffizient nicht mindestens ein Signifikanzniveau von p=0,15 erreichte, wurden ausgeschlossen. Das mathematische Vorgehen war ebenso wie bei der Ableitung der ME-Schätzgleichung.

Bei den Schätzgleichungen für die VQ OM führte die Berücksichtigung der ELOS zu genaueren Gleichungen als die Berücksichtigung der Gasbildung. Ähnlich wie bei der ME-Schätzgleichung war bei Nutzung der ADFom die Schätzgüte höher als bei Verwendung der aNDFom. Von 18 berechneten Regressionsgleichungen zur Schätzung

der VQ OM legt der Ausschuss für Bedarfsnormen die folgende Gleichung fest:

[6]	VQ OM (%)	=	64,45		
		+	0,02677	• ELOS	(g/kg OM)
		–	0,03814	• ADFom	(g/kg OM)

Bestimmtheitsmaß r2: 66 %; Reststreuung sR: 2,44; Schätzfehler sR*100/$\bar{x}$: 3,27 %

4. Gültigkeitsbereich der Formeln zur Schätzung der ME und der VQ OM

Auf Basis der Nährstoffgehalte in dem für die Ableitung der Gleichungen genutzten Datensatz wird folgender Geltungsbereich für die Schätzung der ME und der VO OM angegeben:

ELOS:	325 - 825 g/kg TM
ADFom:	145 - 435 g/kg TM
XL:	5 - 105 g/kg TM
XP:	55 - 105 g/kg TM
XA:	25 - 75 g/kg TM

Falls die Nährstoffgehalte in einer Futterprobe außerhalb dieser Bereiche liegen, nimmt die Genauigkeit der Schätzung ab. Bei der Ausweisung der Ergebnisse ist auf diesen Umstand hinzuweisen.

5. Abschließende Bemerkung

Eine weitere Verbesserung der Schätzgenauigkeiten ist grundsätzlich möglich, wenn zusätzliche Analysendaten berücksichtigt werden können. Der Ausschuss für Bedarfsnormen hält es daher für erforderlich, dass in zukünftigen Verdaulichkeitsversuchen die geprüften Maisprodukte zusätzlich auf die Gehalte an Stärke und Zucker analysiert werden.

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Adresse:
Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie
Eschborner Landstraße 122
60489 Frankfurt am Main

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