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Biotransformation sekundärer Pflanzeninhaltsstoffe

*Kulling S. E., Institut für Sicherheit und Qualität bei Obst und Gemüse, Max Rubner-Institut – Karlsruhe

Sekundäre Pflanzenstoffe sind Pflanzenmetabolite, die im Gegensatz zu den sog. primären Metaboliten für die Pflanze nicht lebensnotwendig sind, in der Pflanze jedoch wichtige Funktionen z.B. als Farb- und Aromastoffe zum Anlocken von Nützlingen oder als Abwehrstoffe gegen natürliche Feinde, wie Insekten, Bakterien und Pilze erfüllen. Der Begriff „sekundäre Pflanzenstoffe“ fasst geschätzt bis zu 100.000 verschiedene Substanzen zusammen, die in Strukturklassen unterteilt werden können. Dazu zählen: Phenolische Verbindungen (z.B. Flavonoide, Phenolsäuren, Stilbene, Xanthone), Isoprenoide (Monoterpene, Phytosterine, Carotinoide, Saponine), Glucosinolate, Alkaloide und Sulfide sowie Phytinsäure.

Werden diese Verbindungen von Mensch oder Tier mit der Nahrung bzw. dem Futter aufgenommen, unterliegen sie in Abhängigkeit von ihrer chemischen Struktur und ihren physikochemischen Eigenschaften nach ihrer Resorption meist einer Metabolisierung durch endogene Phase-I- und Phase-II-Enzyme. Phase-I-Reaktionen, auch Funktionalisierungsreaktionen genannt, sind dabei durch die Einführung oder „Freilegung“ von funktionellen Gruppen gekennzeichnet. So können Hydroxyl-Gruppen durch Cytochrom-P450-abhängige Monooxygenasen eingeführt werden. In Phase II werden die Verbindungen dann durch verschiedene Transferasen in Abhängigkeit von ihrer verfügbaren funktionellen Gruppen mit aktivierter Glucuron-, Schwefel- oder Essigsäure oder mit Glutathion oder Glycin konjugiert mit dem Zweck, die Wasserlöslichkeit zu erhöhen und sie besser ausscheidbar zu machen.

Die körpereigenen Enzyme zur Metabolisierung werden durch das komplexe Enzymspektrum der Darmmikrobiota ergänzt. Im Gegensatz zum körpereigenen Fremdstoffmetabolismus, der in der Regel hydrophilere Stoffwechselprodukte hervorbringt, führen im Rahmen der mikrobiellen Metabolisierung im anaeroben Darmmilieu vor allem reduktive und hydrolytische Reaktionen zu weniger polaren Metaboliten mit niedrigerem Molekulargewicht. Sekundäre Pflanzenstoffe werden dabei vor allem dehydroxyliert, demethyliert, demethoxyliert und/oder hydrogeniert sowie dekonjugiert. Bei vorhandenen heterozyklischen Ringen kann es zudem zu Ringöffnungen kommen. Da die Zusammensetzung der Darmmikrobiota von vielen Faktoren beeinflusst wird und damit variabel ist, zeigen sich bezüglich spezifischer Metabolisierungsreaktionen große interindividuelle Unterschiede.

Im Vortrag werden beispielhaft phenolische Verbindungen, Saponine und Carotinoide, die aufgrund ihrer unterschiedlichen physikochemischen Eigenschaften große Unterschiede in ihrer Verfügbarkeit sowie bezüglich ihrer endogenen und mikrobiellen Metabolisierung aufweisen, besprochen und einander gegenübergestellt. Anhand der Soja-Isoflavone Daidzein und Genistein sowie des Stilbens Resveratrol wird aufgezeigt, welche Faktoren die Verfügbarkeit beeinflussen und auch welche Speziesunterschiede in der Metabolisierung dieser Verbindungen bei Mensch, Ratte, Maus und dem Modellorganismus Caenorhabditis elegans bestehen.

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1.

Post mortem endpoints of ruminal fermentation and anion/proton transporter gene expression as affected by variations in the amounts of physically effective neutral detergent fibre in the diets of growing German Fleckvieh bulls

Zum Einfluss einer variierenden Versorgung von wachsenden Bullen der Rasse Deutsches Fleckvieh auf post mortem Parameter der Pansenfermentation und die Genexpression von Anionen/Protonen Transportern

*Brugger D. , Ettle T., Feser S., Windisch W. M., Bolduan C. – Freising/Poing-Grub

Question: Sufficient alimentary supply with physically effective fibre is crucial for maintaining health and performance of ruminants. Currently, the respective feeding recommendations are re-evaluated with growing German Fleckvieh Bulls (1). In this context, the present study assessed the response of parameters of ruminal fermentation and anion/proton transporter gene expression.

Methods: 70 growing German Fleckvieh Bulls (517 ±32 kg life weight; 314 ±11d of age) were randomly assigned to three dietary treatment groups differing in physically effective neutral detergent fibre (peNDF) (294, 270, 246 g/kg DM) under isoenergetic and isonitrogenous conditions (per kg of DM: 12 MJ ME, 134 g CP) (1). After 191 days of ad libitum feeding, all animals were starved for precisely one day and sacrificed through slaughtering. 20 animals per group were randomly chosen for chemical analyses. Ruminal fluids were analyzed for total short-chain fatty acids (SCFA), ammonia, and pH. Transporter gene expression was quantified by RT-qPCR within total RNA extracts from rumen epithelium. SLC9A3 represents a Na+/H+ antiporter localized in the plasma membrane of rumen epithelial cells. SLC16A1 is a SCFA-/H+ symporter in the basolateral membrane of rumen epithelial cells whereas SLC26A3 is a SCFA-/HCO3- antiporter in the apical membrane. All data was subject to one-way ANOVA.

Results: Reduction of peNDF numerically reduced concentration of SCFAs and, consequently, increased ruminal pH in the lowest supplied group. Ruminal ammonia exhibited a numerically relevant decline within the middle group. The expression of SLC9A3 and SLC26A3 increased with declining dietary peNDF supply. However, these differences were only significant/relevant between the highest and lowest supplied groups. SLC16A1 reacted curvilinear over dietary treatment groups with a significant decline in the middle group compared to the highest and lowest supplied group, respectively.

peNDF/structural value	**g/kg/---**	**294/1.2**	**270/1.1**	**246/0.6**	**SEM**	**ANOVA**
pH	---	7.04	7.06	7.14	0.03	0.06
Total SCFA	mmol/L	55.49	51.30	46.04	3.72	0.19
Ammonia	mmol/L	9.75	8.71	9.25	0.33	0.10
SLC9A3	xfold regulation	1.00^{b}	1.38^{ba}	3.01^{a}	0.12	0.02
SLC16A1	xfold regulation	1.00^{a}	0.70^{b}	1.10^{a}	0.05	0.01
SLC26A3	xfold regulation	1.00^{b}	2.06^{b}	5.68^{a}	0.10	<0.0001

Conclusion: An increased abundance of SCFAs in the rumen is associated with a more efficient absorption of SCFA from the lumen and, conversely, increased transport of HCO_3^- into the lumen in order to buffer the system (2). In the present study, one day of starvation induced a linear decrease in total SCFA with stepwise reduction in peNDF. Consequently, pH in the lowest supplied group was increased. Conversely, the gene expression data points towards a linearly increased expression of SLC26A3 with decreasing peNDF supply. Furthermore, SLC9A3 reacted in a comparable manner to SLC26A3 which indicates a higher necessity to clear protons from the epithelial cytosol in order to stabilize intracellular pH. In summary, we hypothesize reduced peNDF intake induced an increase in SCFA abundance within the ruminal lumen which fostered the necessity for higher $SCFA^-/HCO_3^-$ exchange efficiency at the rumen epithelium in order to stabilize the chemical conditions.

1) Ettle et al. (2016): Proc. Soc. Nutr. Physiol. 25.
2) Aschenbach JR, Penner GB, Stumpff F, Gäbel G. 2011. J. Anim. Sci. 89:1092-1107.

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2.

Ruminal absorption of short chain fatty acids as affected by a continuous or interrupted adaptation to a high concentrate diet in dairy cattle

Einfluss einer kontinuierlichen oder unterbrochenen Adaptation an eine kraftfutterreiche Diät auf die ruminale Resorption kurzkettiger Fettsäuren bei Milchkühen

*Qumar M., Khiaosa-ard R., Pourazad P., Wetzels S., Klevenhusen F., Aschenbach J., Zebeli Q. – Vienna/Berlin

Short chain fatty acids (SCFA) are the microbial fermentation end products and are continuously absorbed through the reticulorumen epithelium to provide energy to the animal. Epithelial adaptation plays a key role in SCFA absorption, but these adaptive changes depend on duration and pattern of high concentrate feeding. The current study was undertaken to investigate the absorption of SCFA at different points in response to the pattern and duration of a high concentrate feeding.

Methods: Eight rumen-cannulated non-lactating Holstein cows were blocked by BW and randomly assigned to two concentrate feeding models, namely continuous or interrupted feeding of high-grain diet. The experiment consisted of two runs (n=8 per model) with washout period of 8 wk. At the start of each experimental run, all cows were fed a forage-only diet (baseline) and gradually transitioned over 6 d to a 60% concentrate diet. Thereafter, cows with continuous feeding of 60% concentrate were kept on this diet for 4 wk. Interruptedly concentrate-fed animals were kept on the 60% concentrate diet for 1 wk, followed by 1 wk of the forage-only diet and then they returned to the 60% concentrate diet for 2 wk. The temporarily-isolated and washed reticulorumen procedure (WRP) was performed (1) at baseline (Base-I; d-0), after the first wk of the concentrate feeding (INT1; d-14) and 2 wk after the concentrate break (INT2; d-35). With continuous concentrate feeding, WRP was performed at baseline (Base-C; d-0) and at the end of the 4-wk challenge (CONT; d-35). During WRP, digesta was removed from the reticulorumen and stored in an insulated container and then the reticulorumen was cleaned with washing buffer. Afterwards, 20-L experimental buffer containing CaCl2 (2 m*M*), MgCl2 (2 m*M*), NaCl (10 m*M*), NaHCO3 (25 m*M*), K2HPO4 (5 m*M*), sodium acetate (60 m*M*), sodium propionate (30 m*M*), butyrate (10 m*M*), and Cr-EDTA (1.8 m*M*) was infused into the reticulorumen of cows for 65 min. The buffer samples were collected at 0 and 65 min for SCFA concentrations (acetate, propionate, butyrate, and total SCFA) and the absorption rates were calculated. Data were analyzed by the mixed procedure of SAS to test the fixed effect of the concentrate periods balanced for values of the respective baseline (co-variable).

Results: The concentrate feeding periods had a strong effect on absorption rates of all SCFA tested ($P<0.01$). The absorption rate of total SCFA during 0-65 min was lowest in INT1 (489 mmol/h), intermediate in INT2 (638 mmol/h) and highest in CONT (845 mmol/h). These values accounted for 90, 118 and 156% of the averaged baseline. Fractional absorption rate of total SCFA during 0-65 min was similarly affected by the concentrate period and that the rates were 23.2, 29.8 and 37.9 %/h in INT1, INT2 and CONT, respectively. Molar proportions of unabsorbed SCFA changed with the incubation time ($P<0.01$) with less propionate and butyrate but more acetate proportions. This pattern of change, particular of acetate and propionate, was more pronounced with CONT than both INT groups.

Conclusions: Highest SCFA absorption was evident after 4 wk of the continuous high concentrate feeding. Therefore, adaptation of the rumen epithelium, as a mechanism to increase the SCFA absorption capability, appeared to require more than 1-2 wk and the interruption of the concentrate feeding postponed the adaptation.

1) ZHANG, S, ALBORNOZ, RI, ASCHENBACH, JR, BARREDA, DR and PENNER, GB, (2013): Journal of Animal Science 91:1685-1695.

This study was supported by Vienna Science and Technology Fund (WWTF).

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3.

Functional and molecular biological evidence for the involvement of TRPV3 and TRPA1 in the absorption of cations by the ruminal epithelium

Funktionelle und molekularbiologische Hinweise auf die Beteiligung von TRPV3 und TRPA1 an der Absorption von Kationen durch das Pansenepithel

*Braun H. S., Hille K. T., Rosendahl J., Aschenbach J. R., Stumpff F. – Berlin

A recent study of the bovine rumen (1) suggests that as previously shown for Na^+ (2), NH_4^+ - induced short-circuit currents are modulated by divalent cations and by menthol and thymol, which are known for their highly selective interaction with three channels of the transient receptor potential (TRP) family: TRPA1, TRPV3 and TRPM8 (3). It was the aim of the present study to identify suitable candidate genes and to study the effect of these agonists on ovine preparations.

Methods: RNA of the ruminal epithelium of three lactating cows and two sheep was isolated and transcribed into cDNA. Intron-spanning primer pairs were used to detect the bovine target genes TRPV1, TRPV2 and the bovine and ovine genes TRPV3-6, TRPA1 and TRPM6-8. To ensure binding to the correct target, all amplicons were subsequently sequenced. The Ussing chamber technique was used to measure the impact of different concentrations of menthol or thymol on the short-circuit current (I_{sc}) and conductance (G_t) of ovine ruminal epithelium (N = 7). Stripped tissues were equilibrated in standard, ammonia-free, bicarbonate-containing buffer solutions gassed with 5% CO_2/95% O_2. Mucosal solutions contained short chain fatty acids (pH 6.4) which were replaced serosally by Na-gluconate (pH 7.4). Menthol, thymol or pure solvent (ethanol) were added to the mucosal side of the tissue, yielding end concentrations of 0, 10, 100 and 1000 µM, with n = 18 tissues in each treatment group. Data were compared using Repeated Measures ANOVA on Ranks.

Results: Ovine and bovine ruminal epithelium expressed mRNA for the following channels: TRPA1, TRPV3, TRPV4, TRPM6 and TRPM7. Only a weak band was discovered for TRPV6, while TRPV5 and TRPM8 could not be detected. No reliable band was found for TRPV1 and TRPV2 in bovine ruminal tissue. In the Ussing chamber experiments, 10 µM menthol or thymol showed no effects versus control. At 1000 µM, menthol (N/n = 4/18) and thymol (N/n = 3/18) had a biphasic effect on I_{sc}, which increased sharply after application of the agonists ($p \leq 0.001$) with a subsequent decline to a value below the original level ($p < 0.05$). Tissue conductance G_t also rose abruptly in response to both agonists and continued to rise after I_{sc} values peaked ($p < 0.001$). At 100 µM, effects of both agonists on I_{sc} and G_t were diverse, with some tissues showing a monophasic and other tissues a full biphasic response.

Conclusion: We present evidence for the expression of mRNA encoding for TRPV3, TRPV4 and TRPA1 in addition to the epithelial Mg^{2+} channel TRPM6 by the ovine and bovine rumen. We confirm expression of TRPM7, while TRPM8 was not expressed. Functionally, we demonstrate that menthol and thymol modulate the I_{sc} and G_t across the ovine ruminal epithelium in a dose-dependent manner. In the absence of a chemical gradient, the increase in I_{sc} should reflect effects of the modulators on transcellular transport. In conjunction with work previously done (1,3), TRPA1 or TRPV3 or both emerge as prime candidates mediating the electrogenic transport of Na^+ and NH_4^+ across the ruminal epithelium, with TRPV4 possibly playing an additional role.

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2) Leonhard-Marek S, Stumpff F, Brinkmann I, Breves G, Martens H (2005) Am J Physiol 288:G630-45
3) Vriens J, Nilius B, Vennekens R (2008) Curr Neuropharmacol 6:79-96

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4.

Challenging the porcine intestinal epithelial barrier function by milk

Effekte von Milch auf die porcine intestinale Barrierefunktion

Radloff J., *Amasheh S. – Berlin

Outline: Newborn's only source of nutrition is milk provided by their lactating mothers. It contains proteins, carbohydrates, fatty acids and other essential nutritive substances which help the newborn thrive and establish a competent immune system. However, some milk components in porcine milk have been identified to have an effect on the epithelial barrier function determined by tight junction proteins, including claudin-5 (1), which might increase absorptive capacity of piglet intestinal epithelium. Thus, we hypothesized that milk has an influence on barrier properties, which might be caused by effects on tight junction protein expression and/or localization.

Methods: Piglet intestinal tissue samples were mounted in conventional Ussing chambers and incubated with a half-and-half mixture of either predigested or non-predigested porcine milk with physiological electrolyte buffer solution on the apical side, while only buffer was used on the basolateral side. Transepithelial resistance was reported, and unidirectional paracellular marker flux analyses were performed using sodium fluorescein under voltage-clamp conditions (0 mV). For further analysis, protein preparations of selfsame tissue specimens were performed, and analyzed by PAGE employing specific antibodies raised against tight junction proteins, including members of the tight junction protein family of claudins.

Results: Both, non-digested and pre-digested milk induced an increase of transepithelial resistance from 39.0 ± 4.6 to 62.0 ± 2.2 Ω • cm2, and from 38.7 ± 4.3 to 46.3 ± 3.7 Ω • cm2 within the first 30 min (n= 6 and 7, ***$p<0.001$ and *$p<0.05$, respectively). Fluorescein marker flux measurements revealed no significant effect on respective paracellular permeability and remained stable during the course of the experiment (3 h, n = 6 and 7, respectively). On protein level, no significant change of claudin-5 was detected after incubation with non-digested and pre-digested milk (n=3, respectively).

Conclusion: Although an increased piglet intestinal epithelial permeability might be a benefit regarding the uptake of nutrients, the opposite effect could be observed in our model. Moreover, no significant changes of a major barrier-determining tight junction protein could be observed.

1) Dittmann I, Amasheh M, Krug SM, Markov AG, Fromm M, Amasheh S (2014). Laurate permeabilizes the paracellular pathway for small molecules in the intestinal epithelial cell model HT-29/B6 via opening the tight junctions by reversible relocation of claudin-5. Pharm. Res. 31: 2539-2548.

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5.

A study of the bovine TRPV3 channel as a candidate protein mediating the ruminal transport of ammonium

Untersuchung des bovinen TRPV3 Kanals als Kandidat für den Transport von Ammonium über das Pansenepithel

*Hille K. T. , Liebe F., Rabbani I., Sponder G., Stumpff F. – Berlin/Lahore

In cattle, large quantities of dietary protein are not utilized but fermentationally degraded to ammonia that is absorbed from the rumen and excreted. At the pH found physiologically in the rumen, uptake of ammonia primarily occurs in the protonated form (NH4+) (1). In conjunction with functional studies using selective modulators (2), expression patterns of mRNA suggest involvement of two members of the transient receptor potential (TRP) family, TRPV3 and TRPA1. However, the permeability of TRP channels to the NH4+ cation has never been investigated. It was the purpose of this study to investigate whether bovine TRPV3 (bTRPV3) channels are permeable to NH4+.

Methods: To induce overexpression of bTRPV3 (XM_005220229), HEK 293 cells were transfected with a bTRPV3Strep-IRES-GFP-vector. The strep-tag serves as a marker for the protein in Western blots; the inserted green fluorescent protein (GFP) allows the identification of successfully transfected individual cells. These cells were selectively investigated via the whole cell and single channel configuration of the patch clamp technique.

Results: In total protein samples from whole cell lysates, detection of the strep-tagged bTRPV3-construct was very strong. Weaker but clearly visible signals for bTRPV3 were demonstrated in the high-purity plasma membrane fraction of overexpressing cells, but not of empty-vector controls. Between 5 and 20% of cells showed a clear GFP fluorescence signal, indicating successful expression of bTRPV3. In whole cell experiments, cells expressing bTRPV3 showed a significant depolarization of the reversal potential in response to application of NH4+, with a rise in inward current at -120 mV. Removal of divalent cations from the solution resulted in a further strong stimulation of inward current at negative pipette potential. Smaller, but clear effects were also observed at +100 mV, suggesting interactions between NH4+ and efflux of Na+. In the single channel configuration and with NaCl in the pipette, single channel events were visible with a conductance of 122 ± 8 pS for Na+ (n = 13) and 143 ± 17 pS for NH4+ (n = 11). Smaller channel events were detectable on occasion. In symmetrical NH4Cl solution, a larger conductance of 240 ± 4 pS (n = 7) was observed.

Conclusion: The bovine analogue of TRPV3 was successfully overexpressed in HEK 293-cells together with GFP. The correct localization of the channel in the plasma membrane was verified. In whole cell experiments, bTRPV3-HEK cells showed a divalent-sensitive conductance with NMDG+ < Na+ < NH4+. On the single-channel level, bTRPV3-HEK cells expressed a Na+ conductance comparable to the human analogue. The conductance to NH4+ was significantly higher. TRPV3 channels may thus play an important role in the uptake of ammonium from the rumen. A variety of other promising channels, in particular TRPA1, but also TRPV4, remain to be investigated in order to unravel the uptake mechanism for NH4+ from the rumen, with possible repercussions for other compartments of the gastrointestinal tract.

1) Abdoun, K., F. Stumpff, et al. (2005). Am J Physiol 289(3): G508-520

2) J. Rosendahl. (2014) Dissertation Thesis, FU Berlin (Berlin).

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6.

Effects of hydrostatic pressure on epithelial cells: approaches to analysis of mechanical force in a mammary gland model, *in vitro*

Effekte von hydrostatischem Druck im Milchdrüsenepithelzellmodell, in vitro

*Mießler K., Markov A. G., Vitzthum C., Amasheh S. – Berlin/St. Petersburg

Outline: In mammary glands, general reorganization and differentiation mechanisms have been shown to affect barrier properties during lactogenesis. Moreover, milk components have been demonstrated to affect epithelial barrier properties (1). As reported recently, during accumulation of milk in murine mammary gland alveolae, a changed expression of tight junction proteins was observed, reflecting a paracellular sealing effect (2). We therefore tested the hypothesis, if hydrostatic pressure alone is able to affect barrier properties in a mammary epithelial cell model, *in vitro*.

Methods: Confluent monolayers of the mammary epithelial cell line HC11 were grown on permeable supports, and were mounted in Ussing chambers, modified with a tube system to provide application of hydrostatic pressure in a range from 100 to 1000 mmH2O. Transepithelial resistance was reported over a time course of 4 hours, during application of 500 mmH2O applied unilateral from the apical side, or 1000 mmH2O bilateral from the basal and the apical side, compared to controls. Selected tight junction proteins were stained afterwards and analyzed with immunofluorescence microscopy. Statistical analysis was performed using Student's t-test, $p < 0.05$ was considered to be statistically significant.

Results: Asymmetric application of pressure was not tolerated by epithelial monolayers. Symmetric application of hydrostatic pressure revealed stable values, but no significant change of transepithelial resistance during a 4 hour-application of 1000 mmH2O, compared to controls (n = 14 and 10, respectively). In accordance, no marked effects on localization of the tight junction proteins occludin and claudin-4 could be detected with immunofluorescence microscopy.

Conclusions: Whereas milk accumulation in mammary glands induces effects on the tight junction barrier of the mammary gland epithelium (2), the single application of hydrostatic pressure does not affect epithelial barrier function under the analyzed conditions, *in vitro*. Therefore, other parameters or milk components may be considered to be responsible for this epithelial barrier sealing effect in mammary glands during accumulation of milk, *in vivo*.

1) Dittmann I, Amasheh M, Krug SM, Markov AG, Fromm M, Amasheh S (2014). Laurate permeabilizes the paracellular pathway for small molecules in the intestinal epithelial cell model HT-29/B6 via opening the tight junctions by reversible relocation of claudin-5. Pharm. Res. 31: 2539-2548.

2) Markov AG, Kruglova NM, Fomina YA, Fromm M, Amasheh S (2012). Altered expression of tight junction proteins in mammary epithelium after discontinued suckling in mice. Pflugers Arch. 463: 391-398.

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7.

Influence of bacterial metabolites on barrier function and pro-inflammatory signalling in epithelial cells *in vitro*

Einfluss bakterieller Metabolite auf die Barrierefunktion und pro-inflammatorische Signale in Epithelzellen in vitro

Villodre Tudela C., Tedin K., Zentek J., *Pieper R. – Berlin

Question: Bacterial metabolites in the intestinal tract may have positive (e.g. butyrate) or negative (e.g. ammonia, amines) influence on host intestinal epithelial cells and barrier function. In a previous study it was shown that, for example, ammonia upregulated pro-inflammatory cytokine and down-regulated the monocarboxylate transporter 1 (MCT1) expression in the colon of pigs *in vivo* irrespective of butyrate concentration (1). The current study was performed to study the influence of different bacterial metabolites and their combination on MCT1 gene expression, pro-inflammatory signalling and barrier function in CaCo-2 cells in vitro.

Methods: CaCo-2 cells between passage 14 and 29 were seed in 24-well plates at a density of 105 cells/well or in Transwell® permeable support filters and applied to 6-well plates at a density of 3x105 cells/well, and allowed to differentiate for 21d. Cells were incubated with increasing concentrations of Na-butyrate (10, 20, 50 mmol), Na-acetate (10, 20, 50 mmol), Na-propionate (10, 20, 50 mmol), Na-lactate (10, 20, 50 mmol), NH4Cl (5, 10, 20 mmol), NaHS (0.5, 1. 2 mmol) putrescine (0.5, 1, 2 mmol), histamine (0.5, 1, 2 mmol), iso-valerate (10, 20, 50 mmol), iso-butyrate (10, 20, 50 mmol) in 3 parallel wells, each. Transepithelial resistance (TEER) was measured in regular intervals over 24 h. After 1 and 24 h, cells from 3 wells were harvested respectively for mRNA extraction. Expression of the MCT1, pro-inflammatory cytokines (TNFα, IL-8), tight junction proteins Occludin (OCLD), ZO1, Claudin-4 (CLDN4), Claudin-2 (CLDN2) was determined using ß-actin and TAT box-binding protein as housekeepers. Expression of MCT1, IL-8 and TNFα was also investigated at 3 different pH levels and three concentrations of Na-butyrate, lactate and ammonia. Co-incubation of increasing amounts of NH4Cl with increasing amounts of Na-butyrate was performed to determine possible amelioration of NH4Cl-induced effects by Na-butyrate. CaCo-2 cells containing a NFkB luciferase reporter gene were seeded in 96-well plates, grown to 80% confluence and used to study the influence of bacterial metabolites on NFkB activation after 4 and 24h, respectively. Data were compared by ANOVA followed by LSD test using SPSS (version 21.0, Chicago, USA).

Results: The TEER values were reduced (P4Cl after 24h and increased values with Na-butyrate (P4Cl, whereas increased expression of OCLD and ZO1 was determined with Na-butyrate. Expression of Il-8 and TNFα was up-regulated in a dose-dependent manner with NH4Cl, Na-butyrate, iso-acids and NaHS. Expression of MCT1 was down-regulated with NH4Cl and up-regulated with Na-butyrate after 24h. Addition of increasing amounts of Na-butyrate to media containing NH4Cl did not ameliorate the negative effects of NH4Cl on pro-inflammatory cytokine and MCT1 expression. However, TEER values remained unchanged when Na-butyrate and NH4Cl were added to the cells together. The luciferase assay revealed that NH4Cl, TNFα and histamine activated NFkB and this was irrespective of Na-butyrate addition.

Conclusions: The data confirm that ammonia and other protein-derived metabolites promote pro-inflammatory cytokine expression through NFkB-mediated signalling in CaCo-2 cells and Na-butyrate did not ameliorate these reactions. This might be explained by cellular starvation under pro-inflammatory conditions due to reduced expression of the MCT1 (2).

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8.

Regulation of the mRNA expression of tight junction proteins in sheep ruminal epithelia by incubation with growth factors *in vitro*

Regulation der mRNA-Expression von Tight-junction-Proteinen im Pansenepithel von Schafen durch Inkubation mit Wachstumsfaktoren in vitro

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The ruminal epithelium of sheep consists of several cell layers. They build an epithelial barrier to limit the entrance of noxes and microorganisms. Epidermal growth factor (EGF) is normally secreted with saliva into the ruminal fluid. It can affect epithelial barrier integrity by altering the expression of tight junction (TJ) proteins in different types of cells (1). Insulin-like growth factor 1 (IGF-1) can also affect TJ in several types of cell monolayers (2), and IGF-1 receptors are present in ruminal epithelia (3). However, despite an expectable role of EGF and IGF-1 on the ruminal epithelial barrier, nothing is known about their effects on the TJ of ruminal epithelia. Therefore, we assessed the effect of the two growth factors on the mRNA expression of TJ proteins at physiological and supraphysiological concentrations after a 7-h incubation *in vitro*.

Methods: Six adult sheep were fed for at least 2 wk with standardized 80% hay and 20% concentrate diet. Thereafter, sheep were stunned and exsanguinated, and ruminal epithelia were harvested from the ventral ruminal sac. Epithelia were stripped off their *tunica muscularis* and *tunica serosa*. Two pieces of epithelium (~3 cm × 3 cm) per sheep and treatment were mounted in Ussing chambers with an exposed area of 3.14 cm2. Epithelial conductance was measured under short-circuit conditions. Incubation buffer on the mucosal side contained short chain fatty acids (SCFA; 40 mM) at pH 6.1; whereas, incubation buffer at the serosal side was SCFA-free at pH 7.4. The control group was incubated for 7 h without growth factors. Treatment groups were incubated for the same period of 7 h with 25 nM or 250 nM IGF-1 on the serosal side, 0.25 nM or 2.5 nM EGF on the mucosal side, or 2.5 nM EGF on the serosal side. After 7 h, epithelia were harvested into RNAlater and changes in the mRNA expression of the TJ proteins claudin-1, -4, -7 and occludin were determined by reverse transcription and subsequent relative quantification by real-time PCR (qRT-PCR). Data were compared using ANOVA with Dunnet post-hoc test and are presented as means ± SEM (n = 12).

Results: The electrical conductance of epithelia treated with the two growth factors at different concentrations was not different to that of the control group. However, the mRNA expression of TJ proteins was affected by growth factors compared with the control group. Application of EGF at 0.25 nM mucosally selectively decreased the mRNA expression of claudin-7 (by -78 ± 3%; $P < 0.05$). A comparable decrease in claudin-7 mRNA expression (by -81 ± 3%; $P < 0.05$) was observed by 2.5 nM EGF serosally; the latter additionally increased the mRNA expression of claudin-1 (by 27 ± 3%; $P < 0.05$). The high concentration of EGF (2.5 nM) on the mucosal side did not affect the mRNA expression of claudin-1 and -7, but selectively decreased the mRNA expression of claudin-4 (by -40 ± 8%; $P < 0.05$). Expression of occludin was not affected by EGF. Serosal application of IGF-1 at either concentration did not affect the mRNA expression of claudin-1 and -4. However, the mRNA expression of claudin-7 was down-regulated (25 nM IGF-1: by -60 ± 4%; 250 nM IGF-1: by -52 ± 7%; $P < 0.05$ each) while the mRNA expression of occludin was up-regulated (25 nM IGF-1: by 167 ± 47%; 250 nM IGF-1: by 223 ± 45%; $P < 0.05$ each).

Conclusion: The presence of EGF on the mucosal side decreases the mRNA expression of claudin-7 at lower and that of claudin-4 at higher concentrations. The effect at lower EGF concentrations at the mucosal side could be mimicked by the effect of higher concentrations at the serosal side, suggesting the receptor presence primarily on the luminal side of the epithelium. The serosal presence of IGF-1 decreased claudin-7 and increased occludin mRNA expression. Collectively, all changes observed at the TJ mRNA expression level did not lead to measurable changes in the tightness of the epithelial barrier.

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9.

Effects of short chain fatty acids on cAMP level as indicator of G Protein activity in sheep ruminal epithelium

Effekte kurzkettiger Fettsäuren auf cAMP - Level als Indikator der Aktivität von G - Proteinen im ovinen Pansenepithel

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It has long been known that short chain fatty acids (SCFAs) play a key role in energy metabolism of ruminants. However, recent studies point to the fact that SCFAs may also serve as signal molecules as being ligands for inhibitory G-protein coupled receptors (GPRs) known as free fatty acid receptors and thereby influencing intracellular pathways. Stimulation of these GPRs mostly inhibits the adenylyl cyclase leading to a lowered intracellular level of 3',5'-cyclic adenosine monophosphate (cAMP), an important second messenger.
The aim of our study was to get a first insight into the functional mechanisms of free fatty acid receptors in ovine ruminal epithelium and their assumed stimulation by SCFAs.

Methods: Stripped ruminal epithelia obtained from ventral sac of sheep were mounted in Ussing chambers. Activation of adenylyl cyclase was triggered by forskolin in the presence or absence of either butyrate or acetate. After incubation, epithelia were separated from subepithelial layers and minced in lysis buffer. Determination of cAMP levels in the supernatant was performed using AlphaScreen cAMP Assay Kit (PerkinElmer). Degradation of cAMP was inhibited by adding 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterase (1), to all buffers. Data were analysed using one-way repeated-measures ANOVA and subsequent Tukey's multiple comparisons test (N=8 sheep).

Results: Forskolin at a concentration of 10 µM led to a 3-fold increase ($p<0.01$) in the level of cAMP in comparison to basal level. In the absence of forskolin, administration of either butyrate or acetate at a concentration of 30 mM did not show any effect on cAMP level. Incubation with butyrate at a concentration of 10 mM or 30 mM for 1 hour following an administration of 10 µM forskolin for 30 minutes lowered cAMP level ($p<0.05$). Administration of acetate tended to lower cAMP level but was not significant.

Conclusion: Forskolin as a direct stimulator of adenylyl cyclase is able to raise the intracellular cAMP level in ovine ruminal epithelium. The SCFAs-mediated decrease of forskolin-induced elevated cAMP level (compared to solely forskolin induction of cAMP) indicates an activation of inhibitory GPRs. Nonetheless, further studies with direct GPRs agonists or antagonists should be conducted to determine whether the lower cAMP levels after SCFAs administration are really due to activation of free fatty acid receptors and not due to indirect effects of SCFAs.

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10.

Investigations on the nitrogen-to-protein conversion factor in organically produced crops

Untersuchungen zum Stickstoff-Umrechnungsfaktor zur Rohproteinbestimmung in ökologisch angebauten Futtermitteln

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The accuracy of the common nitrogen-to-protein conversion factor (factor) of 6.25 for different plant and animal products is recurrently discussed (1, 2, 3). As a result, the factor 5.70 is for example already in use to predict protein content in bread wheat. Variations in the composition of products can lead to differences in the relationship between nitrogen (N) and protein. Therefore, the factor could differ between species and due to breeding progress even within species. Since the composition of organically produced crops can differ from conventional production, it is of interest to check the common factor for different organically produced crops.

Methods: Winter wheat (*Triticum aestivum* L., n=103), blue lupin (*Lupinus angustifolius* L., n=94), spring field pea (*Pisum sativum* L., n=74), and spring field bean (*Vicia faba* L., n=76) samples were collected from organic variety trials in Germany in three years (2011 - 2013). The samples were ground to pass a 1 mm sieve for CP analyses (according to VDLUFA, Kjeldahl N*6.25) or a 0.5 mm sieve for amino acid (AA) analyses. 18 AA were analyzed using HPLC. Assuming that total content of amino acids (TAA) is equivalent to CP content, the recovery was calculated by dividing CP by TAA and the factor by dividing TAA by N (3). Since the molar weight of asparagine (132.12 g/mol) and aspartic acid (133.1 g/mol) as well as glutamine (146.15 g/mol) and glutamic acid (147.13 g/mol) is similar, determining the content of NH3 to estimate amination rate was not necessary for the calculation.

T-tests with approximation for unequal variances (proc ttest cochran, SAS 9.4) were used to compare the factors to the commonly used factor 6.25. Furthermore, the resulting factors for the three species were compared against one another with Kruskal-Wallis-Tests (proc npar1way, SAS 9.4). Pearson correlation analyses (proc corr, SAS 9.4) were conducted to test the relationship between N and TAA.

Results: The determination of CP with Kjeldahl N analyses and the factor 6.25 recovered more than 100 % of TAA. Thus, the factor differed significantly from 6.25 for all four plant species ($p<0.01$). However, high contents of asparagine and glutamine can lead to an overestimation of protein since they contain more N than their acidic derivates. In the table below, CP and TAA contents as well as rates of CP recovery, correlation coefficients, and calculated factors are summarized.

Cultivar	*Crude Protein (N·6.25)*	*Total Amino Acids*	*Recovery*	*r*	*Factor*
Wheat (n=103)	121.8 ± 1.2 g kg-1 DM^{d}	114.2 ± 1.1 g kg-1 DM^{d}	106.7 %	0.97***	5.86 ± 0.02^{b}
Blue lupin (n=94)	322.5 ± 3.7 g kg-1 DM^{a}	310.8 ± 3.6 g kg-1 DM^{a}	103.8 %	0.96***	6.03 ± 0.02^{a}
Field pea (n=74)	212.2 ± 2.5 g kg-1 DM^{c}	206.0 ± 2.3 g kg-1 DM^{c}	103.0 %	0.97***	6.07 ± 0.02^{a}
Field bean (n=76)	295.2 ± 2.0 g kg-1 DM^{b}	263.5 ± 1.7 g kg-1 DM^{b}	112.1 %	0.70***	5.59 ± 0.03^{c}

n=Number of observations, r=correlation coefficient of N to total amino acids (***=$p<0.001$), letters in columns mark significant differences ($p<0.001$)

For organically produced wheat a factor was found that was similar to the one used in human nutrition and significantly lower than for field peas and blue lupins. Field beans had the significantly lowest factor. The prediction of protein based on N seems suitable especially for organically produced wheat, blue lupins and field peas considering very high correlation coefficients between N and TAA. However, the correlation is weaker in field beans maybe resulting of higher contents of NPN like lectins, vicin, convicin, L-DOPA, or nucleic acids.

Conclusions: The results demonstrate that the use of a standardized factor to calculate protein contents from N contents can overestimate protein contents of wheat and grain legumes in organic farming. It should be further discussed to adjust the factor for different species.

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11.

Mathematical description of digestible threonine-response curve in laying hens: A meta-analysis

Mathematische Beschreibung der Wirkungen von Threoninzulagen bei Legehennen: Eine Meta-Analyse

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The three-parameter logistic model has been commonly applied to analyze nutritional response data. This study aimed to derive a generalized form of logistic model (1), namely T model, and apply it to describe digestible threonine (dThr) response in laying hens.

Methods: The generalized model of the form eq [1] with its first and second derivatives eq [3 and 4] was derived (Fig. 1). The derivatives were used to calculate dThr requirements (at the 99% of estimated plateau) and marginal efficiency of utilization. A database with a total of 89 dietary threonine-response treatments was extracted from literature. Threonine values were recalculated and expressed on standardized prececal digestible basis using digestibility coefficients (2). The equation was fitted to the data to describe relationships between dThr intake (mg/hen/day) and egg mass (EM, g/hen/day) of hens.

Results: An acceptable (R2=0.78; RMSE=3.07) fit was obtained when using the model (Fig. 2.a). The fitted dThr-response function is shown in Fig. 1, eq [5]. Using eq [2], the estimated dThr intake needed to produce optimal EM was calculated as 419 mg/hen/day (Fig. 2.a3). The Fig. 2 (b and c) represent the marginal efficiency of dThr utilization. The dThr intakes at the points of maximum marginal efficiency (a2 and b1= 273 mg/hen/day; a1 and c1= 202 mg/hen/day) were calculated using eq [6 and 7] (Fig. 1). The estimates suggest that at the dThr intake of 273 mg/hen/day, the EM is most sensitive to changes in dThr intake level, in the meanwhile, at the dThr intake of 202 mg/hen/day the EM is maximized for the minimum dThr intake.

Conclusions: The presented model was successfully applied to meta-analysis of the dThr intake and laying hens performance. The estimated parameters may be used to calculate nutritional indexes, such as maximum or average response and marginal efficiencies due to graded level of a nutrient. The set of equations (Fig. 1) provides a framework for formulation of nutrient-response relationships.

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$$Y = \frac{A}{1+(-1+\frac{A}{A_0})e^{-\mathrm{Sinh}[bx]+\mathrm{Sinh}[bx_0]}} \quad [1]$$

$$Requirement = \frac{\mathrm{ArcSinh}[\mathrm{Log}[\frac{99e^{\mathrm{Sinh}[bx_0](A-A_0)}}{A_0}]]}{b} \quad [2]$$

$$\frac{dy}{dx} = \frac{be^{\mathrm{Sinh}[bx]+\mathrm{Sinh}[bx_0]}A(A-A_0)A_0\mathrm{Cosh}[bx]}{(e^{\mathrm{Sinh}[bx_0](A-A_0)}+e^{\mathrm{Sinh}[bx]}A_0)^2} \quad [3]$$

$$\frac{d^2y}{dx^2} = \frac{b^2e^{\mathrm{Sinh}[bx]+\mathrm{Sinh}[bx_0]}A(A-A_0)A_0\left(e^{\mathrm{Sinh}[bx]}A_0(-\mathrm{Cosh}[bx]^2+\mathrm{Sinh}[bx])+e^{\mathrm{Sinh}[bx_0](A-A_0)}(\mathrm{Cosh}[bx]^2+\mathrm{Sinh}[bx])\right)}{\left(e^{\mathrm{Sinh}[bx_0](A-A_0)}+e^{\mathrm{Sinh}[bx]}A_0\right)^3} \quad [4]$$

where Y is the response at the nutrient level of x, A is the theoretical maximum value of Y, b is the constant, values of A_0 and x_0 are initial observed values of response and nutrient level, respectively.

$$Y = EM\,(g) = \frac{49.2}{1+2.24\,e^{1.05-\mathrm{Sinh}[0.006\,x]}} \quad (R^2 = 0.78;\ RSME = 3.07) \quad [5]$$

$$\frac{dy}{dx} = \frac{1.9\,e^{\mathrm{Sinh}[0.006\,x]}\mathrm{Cosh}[0.006\,x]}{(6.4+e^{\mathrm{Sinh}[0.006\,x]})^2} \quad [6]$$

$$\frac{d^2y}{dx^2} = \frac{e^{\mathrm{Sinh}[0.006\,x]}((0.08-0.012\,e^{\mathrm{Sinh}[0.006\,x]})\mathrm{Cosh}[0.006\,x]^2+(0.08+0.012\,e^{\mathrm{Sinh}[0.006\,x]})\mathrm{Sinh}[0.006\,x])}{(6.4+e^{\mathrm{Sinh}[0.006\,x]})^3} \quad [7]$$

Fig. 1: Derivation of model (eq [1] to [4]), and parameter estimates (eq [5] to [7])

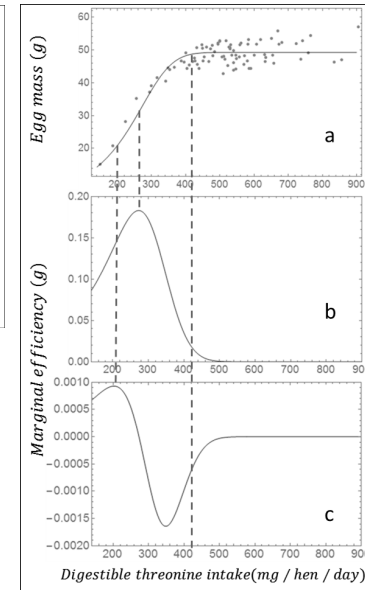

Fig. 2: Response curve to dThr intake (a), with its first (b) and second (c) derivatives

12.

Effect of a low methionine diet on laying performance and egg quality of dual purpose genotypes in comparison to a layer hybrid

Effekt eines Methionin reduzierten Futters auf Legeleistung und Eiqualität von Zweinutzungsgenotypen im Vergleich zu Legehybriden

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Globally, there was a complete decoupling of egg and meat production in the last 60 years which was associated with culling 1-day old male layer cockerels. This practice is controversially discussed in the public and is anticipated to result in legal changes in some countries. As a countermeasure, production systems based on dual purpose genotypes could be established. This approach is facilitated by novel dual purpose genotypes or less specialized, ancient, poultry breeds which may serve a dual purpose. However, a limited performance in laying and fattening is expected, which is unfavourable from a feed efficiency perspective unless a diet of lesser quality can be fed. The objective of the study was to test whether the omission of synthetic methionine (MET) supplementation, which is a prerequisite for organic farming, would be better tolerated by dual purpose than by a layer genotype.

Methods: Four different genotypes were compared with ten individually kept hens each of Lohmann Brown plus (LB, layer hybrid), a novel dual purpose genotype Lohmann Dual (LD) and the ancient dual purpose genotypes Mechelner (ME) and Schweizer Huhn (CH). The hens were from 4 to 6 months in laying. In a cross-over design, each animal received an unsupplemented diet (main components: maize, wheat, rice and soybean meal) and a MET-supplemented diet (+1.6 g dl-MET/kg) for 3 weeks; this in different order. The analysed total dietary MET contents were 4.2 vs. 3.1 g/kg as fed. Both diets were calculated to contain 11.5 MJ metabolisable energy and 168 g/kg crude protein. After 13 d of receiving the respective diet, one egg per hen and treatment was collected and analysed for its general quality. Laying performance and egg weight were recorded daily, body weights weekly. Data were analysed considering genotype, diet, interaction, sequence and, by the repeated statement, hen. Statistical significance for comparisons among means was set to $p < 0.05$.

Results: There were genotype effects (all except one: $p < 0.001$) on hen performance and egg quality traits, whereas no diet effects and interactions were found (Table 1). Body weights (kg) of ME hens were highest (3.4) followed by CH (2.6) and similarly LB (2.0) and LD (1.9). The laying performance was best with LB (98%) and then decreasing in the order of LD, ME and CH (87, 66 and 60%, respectively). Feed efficiency was more favourable in LB and LD compared to CH and ME. All egg weights were in the normal range (> 53 g) in all genotypes. LB and LD laid rounder eggs (shape index 79) than ME and CH (shape index 75) which laid more ovoid eggs. The shell strength (N) was best in LB compared to ME and CH with intermediate values for LD. The Haugh Units, a measure for protein quality in the egg white, were higher in LB and LD than in ME and CH eggs. The ME were superior to the other genotypes in egg yolk proportion, followed by CH, LD and LB whereas the shell proportion in ME eggs was lowest compared to CH, LD and LB.

Table 1: Genotype and diet effects on hen performance and egg quality

	Genotypes				Diets		
Item	LB+	LD	ME	CH	MET-	MET+	SEM
Feed efficiency (g feed/g egg)	1.85^{b}	1.95^{b}	3.56^{a}	3.43^{a}	2.76	2.64	0.202
Shell strength (N)	47.6^{a}	40.6a^{b}	35.5^{b}	35.8^{bv}	39.7	40.1	2.09
Shape index	79^{a}	79^{a}	75^{b}	75^{b}	77	77	0.7
Haugh Units	87.8^{a}	91.1^{a}	77.3^{b}	79.5^{b}	84.1	83.7	1.38
Yolk proportion (%)	25.2^{d}	26.9^{c}	32.4^{a}	29.8^{b}	28.7	28.5	0.44
Shell proportion (%)	9.9^{a}	9.8^{a}	8.5^{b}	9.6^{a}	9.4	9.5	0.21

$^{a-d}$Means within a row carrying no common superscript are significantly different (P

Conclusion: The LB hens performed best followed by LD, whereas ME and CH were least performing. The lower dietary MET content did not affect laying performance and egg quality. At this stage of laying, omitting synthetic MET for 3 weeks was obviously without consequences for any genotype. All eggs were of a high quality. An interesting result was the rounder egg shape of LB and LD compared to the ancient dual purpose breeds ME and CH.

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13.

Nitrogen and sulfur containing amino acid concentration in feather and feather-free body protein of fast growing meat type broiler chicken dependent on gender and age

Gehalte an Stickstoff und schwefelhaltigen Aminosäuren im Feder- und federfreien Körperprotein von Masthähnchen in Abhängigkeit von Geschlecht und Alter

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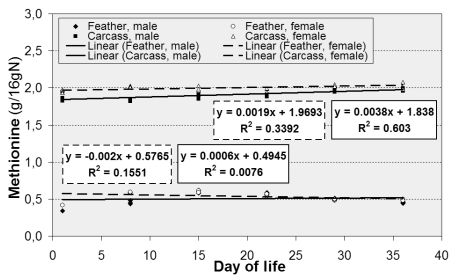
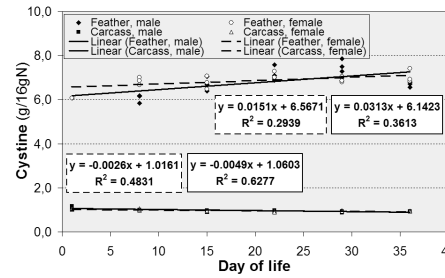
In poultry diets, sulfur containing amino acids (SAA) are in focus according to their considerable contribution to feather protein which contains a high content of cystine (Cys) but low methionine (Met) concentration. In contrast, more Met than Cys was observed in the feather-free carcass [1-4]. Therefore, differences in feathering rate of broiler chicks may affect the dietary Cys and Met needs. Due to very scarce amino acid (AA) composition data for feather and body proteins of current genotypes, the hypothesis of the present study was if AA composition data depending on gender and age of modern fast growing meat type broiler chickens are modified.

Methods: Two growth experiments with 180 male and female (1:1) broiler chickens (ROSS 308) were conducted. Both the starter (d1 to d22) and grower diet (d22 to d36) based on a constant mixture of corn, wheat, soybean meal, soybean protein concentrate and crystalline feed AA were formulated to provide nutrient contents according to current recommendations. The dietary AA composition was adjusted near to ideal AA ratios [5]. Day old birds were divided into 30 floor pens of 5 chicks per pen and 15 pens per gender and reared on the experimental diet. Additionally, 15 day old male and female chickens (each 3 samples of 5 chicks) were euthanized by CO2inhalation following 24h fasting. Afterwards, the feathers were manually removed and the extent of feathering was measured by weighing. Every week the same procedure was applied for 15 individual chickens per gender (3 pens of 5 birds, respectively). In the feather and feather-free carcass the N and AA contents were determined. Regression analysis and one-way ANOVA (SPSS software package) connected with Tukey-test were utilized to identify significant differences between variables (p

Results: The relative feather proportion increased non-linearly with elevated body weight of chickens and was significantly higher in female *vs.*male birds. The feather and carcass N content decreased with increasing age (p





Conclusions: According to the observed variation of Met and Cys content in the feather and featherless carcass protein, effects on the optimal dietary Met to Cys ratio dependent on gender and age of growing chickens are expected. These factors need more attention in requirement studies namely when based on factorial approaches.

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14.

The effect of different dietary methionine levels on the growth performance, antioxidant status and tissue biochemical parameters of turkeys

Der Einfluss unterschiedlicher Methioningehalte im Alleinfutter auf Wachstumsleistung, Oxidationsstatus und biochemische Gewebeparameter bei Puten

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The aim of this study was to investigate whether dietary supplementation with graded levels of methionine (Met) in the final stage of rearing (9 – 16 weeks) affects the antioxidant status of turkeys and turkey meat.

Methods: A total of 630 eight-week-old female Hybrid Converter turkeys were divided into 6 groups (with 7 replicates per group and 15 birds per replicate in each) and were fed the same basal diet without (group 1) or with different addition of DL-methionine (groups 2 – 6). The total Met content of diets in experimental groups was as follow: 2.9/2.4, 3.2/2.8, 4.0/3.4, 4.7/4.2, 5.6/4.7, and 6.1/5.5 g/kg, at 9 - 12/13 - 16 weeks of age, respectively. In basal diets content of Met+Cys was: 6.4/5.7 g/kg, respectively. In both feeding phases, dietary Met levels in groups 3 and 6 corresponded to those recommended by NRC (1994) and BUT (2012), respectively. The growth performance of birds and blood biochemical parameters were analyzed in all groups (7 samples from each group), whereas the indicators of the antioxidant status in the liver and breast muscles were determined on samples representing groups 1, 3 and 6 (one from each of the 7 replicates).

Results: The varied concentrations of methionine had no significant effect on the growth performance of turkeys, including final body weights and carcass dressing percentage. The Met content of diets influenced selected parameters of the blood redox status of turkeys. Based on plasma total antioxidant capacity (FRAP), experimental groups could be arranged in the following order: 5 > 6 > 4, 3, 2, 1, with a significant difference between groups 4 and 1.

Table 1. Selected parameters of the redox status in the liver and meat of turkeys

	Met level, g/kg			SEM	P
	2.9/2.4	4.0/3.4	6.1/5.5		
Liver					
SOD, U/g of protein	11.6[a]	10.0[b]	9.59b	0.288	0.003
GSH/GSSG ratio	2.97[a]	3.13[a]	0.47b	0.308	0.001
MDA, µmol/kg	1.03[a]	0.76b	0.92[a]	0.038	0.005
Breast muscle					
Catalase, U/g of protein	12.1[a]	10.2[b]	9.48[b]	0.331	0.001
SOD, U/g of protein	3.15	3.17	3.24	0.090	0.926
GSH+GSSG, µmol/kg	4.06[b]	5.15[a]	4.66[a]	0.154	0.003
MDA, µmol/kg	1.37	1.31	1.15	0.038	0.062

GSH - reduced glutathione; GSSG - oxidized glutathione

superoxide dismutase (SOD) activity in the liver was similar in groups 3 and 6. The ratio of reduced glutathione to oxidized glutathione was significantly higher in groups 1 and 3, relative to group 6. In comparison with group 3 birds, group 6 turkeys were characterized by higher malonyl dialdehyde (MDA) levels. No significant differences were observed between groups 3 and 6 in the redox status of breast meat. In comparison with those groups, the meat of group 1 turkeys was characterized by higher catalase activity, lower glutathione concentrations and higher MDA levels.

Conclusions: In the final stage of rearing, between 9 and 16 weeks of age, different dietary Met levels had no significant effect on the growth performance of turkeys. Increasing dietary Met concentrations above the levels recommended by NRC (1994), led to an increase in the antioxidant capacity of blood, but it did not improve the analyzed indicators of oxidation-reduction processes in the liver and meat.

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15.

Evaluation of *Hermetia illucens* and *Spirulina platensis* proteins in semi-synthetic diets for the laboratory rat

Bewertung von Hermetia illucens und Spirulina platensis Proteinen in halbsynthetischen Diäten für die Laborratte

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Currently, alternative protein sources are in special focus of animal nutrition to lower the demand for import proteins like soybean meal. Two of the candidates are larvae of the black soldier fly, *Hermetia illucens* (HI), and the blue green algae *Spirulina platensis* (SP*)*. Both protein sources have a high protein content and an adequate amino acid composition, therefore they are in the field of very promising alternative protein sources. The aim of this study was to evaluate both the acceptance and dietary protein quality of partly defatted HI larvae and *Spirulina platensis* in nitrogen balance studies with laboratory rats.

Methods: 30 male juvenile Wistar rats (RccHan:Wist, Harlan Laboratories GmbH) were used in a N-balance trial. The experiment consisted of 2 consecutive collecting periods. The rats were divided into six experimental groups. The main ingredients in the diets were wheat starch, cellulose, sucrose, and soybean oil. The diets contained 19% *Spirulina* meal or 26% HI meal (partly defatted) in order to achieve approximately 15% crude protein in the final diets. Because of the powdery consistency of *Spirulina* meal, the protein source was applied in 1:1 mixture with corn meal. Additionally, diets with both protein sources were supplemented with the expected first and second limiting amino acid (Met and Lys for SP diets; Met and Thr for HI diets). Faeces and urine were quantitatively collected and analyzed for their nitrogen content according to standard procedures. Apparent N-digestibility (appND), biological value (BV) and the standardized net protein utilization (PNUstd) were calculated according to (1) by application of an exponential N-utilization model (2). Statistical analysis (ANOVA, Twwukey-test, Games-Howell-test) was conducted by SPSS.

Results: The semi-synthetic diets were accepted by the rats, independent on the protein source under study. The results of the experiments are summarized in the table. The apparent nitrogen digestibility was higher than 80% in all diets, but *Hermetia* diets tended to yield higher appND data. Otherwise, *Spirulina* diets led to increased BV and PNUstd, respectively. The amino acid (AA) supplementation of both basal diets provided inconsistent results. Supplementation of *Spirulina* diets with the expected limiting amino acids did not improve the protein quality parameters. Otherwise, a strong effect was observed with the diet *Hermetia*+Met, but Met and additional Thr supplementation yielded no improvement of dietary protein quality as compared to the basal diet. This observation was unexpected and difficult to explain.

Diet	Spirulina	Spirulina +Met	Spirulina +Met+Lys	Hermetia	Hermetia +Met	Hermetia +Met+Thr
appND [%]	$81.2^{a} \pm 1.0$	$81.8^{ab} \pm 1.3$	$81.6^{ab} \pm 1,5$	$82.0^{ab} \pm 1.3$	$83.1^{b} \pm 2.1$	$82.3^{ab} \pm 0.7$
BV [%]	$65.1^{c} \pm 3.0$	$67.5^{c} \pm 3.4$	$66.1^{bc} \pm 7,9$	$55.5^{a} \pm 4.6$	$69.7^{c} \pm 5.4$	$57.8^{ab} \pm 5.3$
PNUstd [%]	$57.4^{b} \pm 2.7$	$58.8^{b} \pm 2.8$	$58.0^{ab} \pm 6,9$	$50.0^{a} \pm 5.7$	$62.4^{b} \pm 5.4$	$51.4^{a} \pm 4.4$

Means (± SD); appND = apparent N-digestibility; BV = biological value; PNUstd = standardized net protein utilization (standardized N-intake = 1100 mg/BWkg0.67); different superscript letters reveal significant differences between diets ($p<0.05$).

Conclusion:

The results show that, both partly defatted *Hermetia* meal and *Spirulina* powder in mixture with corn meal are promising novel protein sources, but inconsistent reaction on AA supplementation indicates that additional research is needed for further improvement of AA balance for further enhancement of protein quality of these alternative protein sources.

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16.

Nitrogen and methane excretion of cows fed a low crude protein diet with too low content of utilisable crude protein from calving onwards

Stickstoff- und Methanausscheidung von frisch-laktierenden Kühen bei Verfütterung einer Ration mit niedrigem Rohproteingehalt und zu geringem Gehalt an nutzbarem Rohprotein

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In order to abate the environmental problems caused by N emissions from milk production, there are ongoing attempts to avoid any excessive crude protein (CP) supply to the cows (1). For approaching the threshold best, the requirements for utilizable CP (uCP) have to be known. However, directly after parturition the true milk production potential remains unknown and a deficiency may go unnoticed. Such a deficiency in uCP, along with CP reduction, might even result in a secondary energy deficiency as the response of the farmers would be to cover energy and nutrient supply on basis of the requirements estimated from actual yield of the cows. However, it is unclear if the CP reduction in this case is at least effective in mitigating N emission intensity (emissions per kg of milk produced). The feeding measure investigated may also have consequences for methane emissions, because feed allocation and milk yield are affected.

Methods: Two diets were fed from the day of calving to 2×9 dairy cows, which were similar in yield across the previous lactation. The analysed CP contents in the deficient and control diets were 13.2 and 14.7% in dry matter, respectively. The deficient cows ingested 26% less CP than the control cows. The amounts of feed were weekly adapted to the actual milk yield. This was associated with a calculated deficiency of uCP by 15% relative to actual milk yield. During each of the 8 weeks following calving, samplings and assessments of intake and excretion with faeces and urine were performed for 5 days. For the remaining 2 days the cows were put into open circuit respiration chambers for determination of methane emissions. The cows were weighed weekly. Data were subjected to analysis of variance with a repeated measurement statement. Treatment, week and the interaction were considered as effects.

Results: Across the 8 weeks, the true levels of energy and protein deficiency in the deficient cows, as estimated from the performance (milk yield and body weight) of the control, were 14 and 33%, respectively. Relative to control, the deficient cows consumed 18% less dry matter and produced 25% less milk (26 vs. 34 kg/d). The proportion of dietary N utilised for milk N synthesis was the same in both groups as were proportionate N losses with the excreta. Urine N proportion of total excreta N did not differ with 31 and 33% in the deficient and control cows, respectively. Together this caused that the N emission intensity per kg milk N was similar between treatments. The deficient cows emitted 22% less methane. Also this resulted in similar methane yields (18.2 v. 18.9 g/kg dry matter intake for deficient and control cows) and emission intensities in both groups. Although there were some week effects, there was no significant interaction with week in any of the response variables described.

Conclusion: Even though the 26% reduction in dietary CP intake was helpful for reducing absolute N and methane emissions, the associated deficiency in supply with uCP, coupled with a too low supply with energy, rendered this feeding measure inefficient in terms of reducing N and methane emission intensity. Also the level of potential gaseous N emissions from the manure should not have differed when related to milk production due to the similar urine N proportion of total manure N found. It is even likely that, at the time when body stores have to be replenished, a compensatory increase in emissions takes place because of the higher intake of N and fermentable organic matter needed to accomplish this. Therefore, it is highly advisable, also from an animal welfare point of view, to ensure that uCP requirements are covered from the beginning of lactation when introducing low-CP diets.

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17.

Effects of enzyme substitution on endogenous N losses (praecaecal and total) in pancreatic duct ligated minipigs fed a N free diet

Effekte der Enzymtherapie auf die endogenen N- Verluste (praecaecal und gesamt) pankreasgangligierter Minipigs bei Einsatz einer nahezu N-freien Diät

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The pancreatic duct ligated pig (PL) is an established model for pancreatic exocrine insufficiency (PEI). Loock (2010) found increased praecaecal (pc) and total losses of endogenous (end) N in PL-pigs. The higher amount of chyme (increase of dry matter [DM] flux by factor 6.76 compared to controls) due to the reduced digestibility was assumed to be the main reason for that finding. The lack of pancreatic enzymes in case of PEI results in maldigestion of nutrients and end N; furthermore higher end N losses might stimulate the microbial protein synthesis in the hindgut. Aim of this study was to test the effects of pancreatic enzyme substitution (PES) on end N losses in PL-pigs.

Methods: Overall 11 adult minipigs with an ileo-caecal re-entrant fistula were used. In 7 pigs the pancreatic duct was ligated (PL), 4 sham operated pigs served as controls (C). All pigs were fed a complete diet (36.3 g N/kg DM), before switching to the almost N-free diet (0.544 g N and 829 g starch / kg DM) based on corn starch. Animals were fed 222 g DM twice a day. PL-pigs were fed the N-free diet twice: without (PL-0) and with addition of PES (PL+enz; accounting for 0.09 g N; ~47000 IU Lipase, 52000 IU amylase, 3300 IU protease) with each meal. Chyme collection was performed on 7 consecutive days over 12 h. In an additional trial, feces were collected over 10 days. N-content was analyzed according DUMAS in chyme and feces. Statistical analysis was done using ANOVA and student`s t-test.

Results: PEI resulted in a significant increase of ileal chyme; PES caused a reduction of chyme mass but values were still markedly higher than in C. The DM-content of ileal chyme was 3-times higher in PL-0 and more than doubled in PL+enz compared to C. Although N-content was lower in chyme of PL-pigs the ileo-caecal N-flow was at least two times higher than in C, even PES was done. For fecal mass there was only a tendency for higher DM mass in PL-0. PEI resulted in markedly higher end fecal N-losses, which were not affected by PES.

Table 1: Chyme and feces mass (fresh mass (FM) and DM), N-content and total endogenous N in controls, PL-pigs without or with PES

	Chyme (day 5 of collection)			Feces (day 10 of collection)		
	Controls (n=4)	PL-0 (n=7)	PL+enz (n=7)	Controls (n=4)	PL-0(n=3)	PL+enz (n=3)
FM (g/24 h)	$778^{A} \pm 244$	$1128^{Aa} \pm 226$	$1024^{Ba} \pm 316$	72.7±16.0	111±26.6	93.9±27.5
DM content (%)	$9.17^{A} \pm 1.52$	$30.5^{Ba} \pm 4.87$	$21.8^{Bb} \pm 6.09$	42.2±4.60	39.2±0.220	39.0±2.40
DM (g/24 h)	$74.4^{A} \pm 38.2$	$340^{Ba} \pm 64.8$	$234^{Ba} \pm 106$	30.1±4.50	43.5±10.2	36.4±10.2
N (g/kg DM)*	$42.9^{A} \pm 13.7$	$17.6^{Ba} \pm 0.470$	$24.8^{Ba} \pm 8.86$	12.6±1.55	38.74±9.47	40.3±9.23
N (g/24 h)*	$1.42^{A} \pm 0.198$	$2.96^{Aa} \pm 0.912$	$2.64^{Ba} \pm 1.29$	0.379±0.080	1.70±0.565	1.48±0.530

Different capital letters mark significant differences between controls and PL-pigs; small letters mark significant differences between PL-0 and PL+enz. (t-test). For calculation of daily ileo-caecal N-flux the amount collected within 12 h was doubled. *For calculation of losses via feces the composition of feces of day 10 was used; to minimise effects of days without feces dropping mean of day 1-10 was used for feces amount.

Conclusion: Experimentally induced PEI resulted in a distinct increase of mass of ileal chyme. The use of PES did not normalize neither the mass of ileal chyme nor pc end N losses. The massive postileal digestion of the diet consisting mainly of starch (> 300 g / 24 h) is noteworthy as it indicates that high rate of microbial fermentation and protein synthesis took place in both groups. Comparing the amount of end N in ileal chyme and feces shows a marked postileal absorption of N in C (~ 73 %), while this rate was much lower in PL-pigs (43 % in PL-0; 44 % in PL+enz). Nonetheless absolute amount of N disappearing from digesta during hindgut passage was similar in PL+enz and PL-0. N is absorbed from the hindgut mainly in form of ammonia without any nutritive value. Praecaecal and fecal end N losses were not affected by PES. Whether a normalisation can be achieved by higher dosed PES needs further investigation. To conclude PEI results in higher end N losses despite PES (factor 3.9 on fecal basis). Therefore higher protein supply is recommendable in these patients even when treated with PES.

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18.

Chemical whole body composition of female chickens of four genetically diverse purebred layer lines reared with increasing dietary L-arginine

Ganzkörperzusammensetzung weiblicher Hühner vier genetisch divergenter Reinzuchtlegelinien in Abhängigkeit steigender L-Arginin-Konzentration im Futter

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L-arginine (Arg) is dietary indispensable for chickens. Among others, the Arg requirement for optimal body growth depends on chickens' genetically determined growth potential. In its function as precursor of body proteins, multifunctional nitric oxides and growth-promoting polyamines, Arg is capable of modulating several pathways of the protein and lipid metabolism. Therefore, the effects of an increasing dietary Arg supply on the chemical whole body composition of female chickens of four layer lines differing in phylogeny and performance (WLA/R11: high/low performing white layers; BLA/L68: high/low performing brown layers) were examined from hatch to 18 weeks of age.

Methods: 36 one-day-old female chicks of each line were distributed to three diets providing 70 (LA), 100 (AA) and 200 % (HA) of recommended Arg supply (NRC, 1994; GfE, 1999) under *ad libitum* feeding conditions. From week 7 to 18 birds were fed with corresponding Arg diets for pullets. Residual feed was recorded weekly. From hatch to 18 weeks of age three chicks of each group were weighed and bloodless killed by CO2 in six-week-intervals. The ingesta were removed from birds' digestive tract totally. These emptied bodies including feathers were frozen at -20°C and ground. Thereafter homogenized bodies were freeze-dried over 48 hours and analysed for dry matter, crude ash and crude protein in accordance to the VDLUFA methods. Crude fat was calculated by the difference between organic matter and crude protein. The statistical evaluation of data was performed with a 4 x 3 x 4 three-factorial ANOVA (line, diet and age) by using Tukey-Kramer test in SAS procedure MIXED. LSMeans differences were considered to be statistically significant for $p<0.05$.

Results: Brown layer lines showed higher emptied body weights with a higher relative ash content than white lines ($p<0.001$; Table). Whereas chicken bodies showed no genetic impact on the relative lipid content, the relative protein content of R11 was higher than that of the other lines ($p<0.001$). In addition to feed consumption and emptied body weight, the relative content of ash, protein and lipid increased age-dependently in chickens ($p<0.001$). In contrast to the adequate and surplus dietary Arg supply, deficient dietary Arg depressed feed consumption and body growth from week 6 onwards ($p<0.001$) and elevated the relative lipid content of 18-week-old brown layers ($p<0.01$).

Conclusions: The rearing of layer-type chicken comprises a period of body growth and its chemical composition, whose quality strongly depends on chicken's phylogenetic origin and adequate nutrient supply. In this period deficient dietary Arg serves as source of metabolic variation between brown and white layer lines. Although Arg does not influence chickens' protein content in this study, the Arg deficiency seems to intensify the metabolic redistribution of nutritional energy from the accretion of proteins to those of lipids.

	Line (L)	Hatch			Week 6			Week 12			Week 18			PSEM
		LA	AA	HA	LA	AA	HA	LA	AA	HA	LA	AA	HA	
Emptied body weight (EBW, g)	WLA	34	35	34	241	312	289	667	743	843	898	962	1015	15.5
	BLA	37	37	37	243	280	308	697	775	747	794	848	941	
	R11	33	34	33	218	242	197	566	597	574	704	862	878	
	L68	38	38	38	263	362	275	804	955	740	1156	1148	1082	
Lipid content (% of EBW)	WLA	9.7	9.8	9.9	10.8	10.0	11.1	10.4	11.5	14.2	11.5	12.3	12.1	0.7
	BLA	7.6	7.7	7.8	14.9	12.4	9.4	13.3	13.3	11.2	12.2	9.7	10.6	
	R11	8.0	8.1	8.1	11.9	11.0	11.2	14.1	11.7	11.7	10.2	12.3	11.9	
	L68	9.2	9.4	9.3	12.1	9.9	9.1	12.5	14.9	12.0	15.5	12.7	11.7	
Protein content (% of EBW)	WLA	12.0	12.2	12.1	19.1	19.2	18.4	20.8	20.8	20.0	21.7	21.8	21.8	0.4
	BLA	13.4	13.5	13.7	18.0	18.0	19.4	19.4	20.2	20.5	21.9	22.2	21.9	
	R11	15.3	15.3	15.3	18.4	19.9	19.0	20.4	21.6	21.2	23.1	22.3	22.0	
	L68	14.7	14.8	14.9	18.7	19.7	18.8	18.7	20.7	20.8	21.5	20.8	22.0	

ANOVA (p values)	Line	Diet	Age	L x Diet	L x Age	Diet x Age	L x Diet x Age
EBW	**< 0.001**	**< 0.01**	**< 0.001**	0.097	**< 0.001**	0.335	0.622
Lipid content	0.164	**< 0.05**	**< 0.001**	**< 0.01**	**< 0.001**	0.175	**< 0.05**
Protein content	**< 0.001**	0.067	**< 0.001**	0.169	**< 0.001**	0.087	0.143

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19.

Variation of amino acid digestibility of maize grains from different genotypes in caecectomised laying hens

Variation der Aminosäurenverdaulichkeit von Maiskörnern unterschiedlichen Genotyps bei caecectomierten Legehennen

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Maize grain is a common ingredient in diets for laying hens. Maize grain mainly serves as an energy source, but considerably contributes to the amino acid (AA) supply at high inclusion level in the diet. Considering AA digestibility (AAD) in feed formulation is an effective tool to increase the efficiency of protein utilization in laying hen feeding. It was the objective of this study to investigate the variation of AAD of maize grains and to evaluate approaches to predict AAD of maize grains.

Methods: Maize grains from 20 different genotypes with crude protein (CP) concentrations ranging from 78 to 112 g/kg DM were used. Grains were characterised by physical properties (thousand seed weight, test weight, extract viscoelasticity), chemical composition (proximate nutrients, fibre fractions, starch, AA, minerals, inositol phosphates) and gross energy concentration. Additionally, the *in vitro* solubility of nitrogen (N) after pre-treatment with pepsin and pancreatin was determined. Caecectomised LSL-Classic hens were individually housed in metabolism cages at the age of 56 weeks. The experimental design comprised a 6x6 Latin Square in fourfold replication. The 6 hens within one Latin Square were either fed a basal diet containing 500 g/kg maize starch or one of 5 maize diets, each containing a maize genotype at the expense of maize starch. Hens were offered 120 g/d of the respective diet and refusals were recorded. After a 4-day adaption period, excreta were collected quantitatively for 4 days. A linear regression approach [1] was used to determine the AAD of the maize genotypes (Proc MIXED, SAS 9.3). Correlation analysis (Proc CORR) was used to study relationships between AAD and analysed fractions of the genotypes. Multiple linear regression analysis (Proc REG) was performed in an attempt to explain the variation in AAD. Variables entered the regression model when when significant ($P < 0.10$). Equations were evaluated by using the adjusted R^2 and the RMSE values.

Results: The AAD was significantly ($P < 0.05$) influenced by the genotype. Among the essential AA, the lowest mean digestibility was found for Trp (69%) and the highest for Leu (94%) (Table). The digestibility of Lys and Met ranged from 64-85% and 86-94%, respectively. With a difference of 66 percentage points the largest variation was observed for Trp. Significant correlations ($P < 0.05$) between the CP concentration and AAD of several AA were found, including Lys (r=0.50), Met (r=0.47) and Thr (r=0.50). Other significant correlations between AAD and physical and chemical characteristics were found only in few cases and without a consistent pattern among AA. The *in vitro* solubility of N was not significantly correlated with AAD. The accuracy of all equations predicted to estimate AA digestibility varied between AA but was only on an overall low level.

Table: Grain amino acid digestibility (%) of different maize genotypes (n=20)

	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Mean	90	89	87	88	94	79	91	91	83	69	89
SD	1.9	3.0	2.9	3.6	1.4	4.5	1.9	1.9	4.2	14.3	3.0
Min.	85	80	80	78	89	64	86	85	72	21	80
Max.	94	93	92	93	96	85	94	95	89	88	94

Conclusions: The AAD of maize grains from different genotypes varies significantly in laying hens. Chemical and physical characteristics analysed in this study were not adequate to predict the variation in AA digestibility with good accuracy. Further studies are necessary to develop tools that allow for consideration of the variation in feed formulation based on digestible amino acids.

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20.

Intravenous administration of L-arginine during late-pregnancy influences ovine milk composition in the early post-partum period

Intravenöse Gabe von L-Arginin in der Spätträchtigkeit beeinflusst die ovine Milchzusammensetzung in der Frühlaktation

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A critical development phase of the ruminant mammary gland is during pregnancy. During this time frame the number, size and milk production potential of secretory cells increases. At parturition, the numbers of secretory cells in the mammary gland are close to their maximum complement, while secretory cell milk production potential remains plastic. Thus, pregnancy provides an opportune time frame to develop intervention strategies aimed at increasing subsequent lactation performance. L-arginine has been shown to improve mammary gland function and development in a number of species. Thus, we examined whether intravenous administration of L-arginine to twin-bearing Romney ewes during late-pregnancy influences mammary gland development and function.

Methods: Two cohorts of twin-bearing Romney ewes from day (d) 100 to d 140 of pregnancy (Cohort 1) or d 100 to parturition (Cohort 2), received an intravenous bolus of either L-arginine-mono-hydrochloride (345 µmol/kg body weight) or approximately the same volume of sterile saline three times daily (0800, 1600, 2400 hrs). At d 140 ewes from cohort 1 were euthanized and maternal mammary tissue collected for analysis of total DNA, RNA, protein and the abundance of total and phosphorylated (Ser2448) mechanistic target of rapamycin protein. Differences between treatment groups were determined using the MIXED procedure in SAS that included the fixed effect of dam treatment group. Cohort 2 ewes were allowed to naturally lamb and milk was collected over a 14 day period (d 1, 4, 7, 10 and 14) to assess milk yield and composition. Daily milk yield and composition was calculated using repeated measures analysis and the MIXED procedure in SAS, with a linear model that included the fixed effects of dam, dam treatment group, and the covariates average dam live weight, average dam body condition score and days in lactation.

Results: In cohort 1, total mammary DNA content (cell number) tended to be higher ($P = 0.07$) in ewes intravenously administered L-arginine. However, no change was observed in total RNA, protein, or measures of protein synthetic efficiency (Protein:RNA), cell size (Protein:DNA) and protein synthetic capacity (RNA:RNA). In addition, the abundance of total mTOR and mTORSer2448 protein was unaffected. In cohort 2, milk composition analysis from ewes intravenously administered L-arginine showed higher crude protein percentage at d 7 ($P = 0.02$) and d 10 ($P = 0.04$), but tended to be lower at d 14 ($P = 0.07$). A weak trend for higher fat percentage was observed in control ewes at d 1 ($P = 0.10$), compared to treated ewes. No effect on lactose percentage, milk, protein, fat or lactose yield was observed.

Conclusion: Intravenous administration of L-arginine during late-pregnancy appears to have a limited effect on the development of the pre-partum mammary gland in twin-bearing Romney ewes, with only a weak trend for increased DNA content observed. However, in the immediate post-partum period there was a transient increase in milk protein percentage suggesting a change in the protein synthetic machinery post day 140.

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21.

Amino acid composition of grass silages containing different levels of true protein in total crude protein

Aminosäurezusammensetzung von Grassilagen mit unterschiedlichen prozentualen Reineiweißgehalten im Rohprotein

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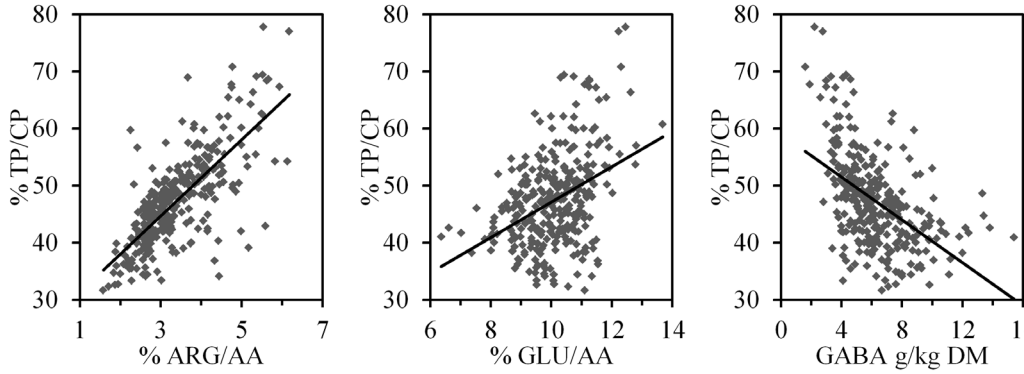
It is well known that after harvesting grass for silage production, plant enzymes like proteases degrade the true protein (TP). As long as the grass is not wilted yet, enzymes in the plant cells can still function, hence the percentage of TP in total crude protein (CP) decreases. Simultaneously, the amino acid (AA) composition of the grass changes. 311 grass silages were analyzed for their contents of AA, TP and CP. The aim of the present study was to investigate whether the AA profile changes with different percentages of TP in CP.

Methods: The AA composition of all 311 grass silages was analyzed via VDLUFA III, method 4.11.1 by Evonik Nutrition & Care GmbH, Hanau, Germany. Assayed AA were: MET, CYS, LYS, THR, ARG, ILE, LEU, VAL, HIS, PHE, GLY, SER, PRO, ALA, ASP, GLU as well as the biogenic amine GABA (γ-aminobutyric acid). The Institute for Animal Nutrition, University of Veterinary Medicine Hanover Foundation, analyzed TP and total CP contents. TP contents were determined using the BARNSTEIN method, corresponding to VDLUFA III, method 4.4.1. The amount of total CP was analyzed via KJELDAHL according to VDLUFA III, method 4.1.1. The concentration of every single assessed AA was converted into a percentage of the sum of all measured AA. Statistics were evaluated via Spearman rank correlation (src).

Results: Statistics show a highly significant correlation ($p < 0.001$) between the contents of arginine (src = 0.73), glutamate (src = 0.36), GABA (src = -0.52) and the respective percentages of TP in CP in the grass silages, as can be seen in Figures 1-3. The other AA had no or only low correlations.



Correlation between arginine (Fig. 1, left), glutamate (Fig. 2, center) and GABA (Fig. 3, right) and the percentage of TP in total CP

Conclusion: Contradicting to literature, a change in AA concentration was only noticeable for arginine, glutamate and GABA. Deficiencies of arginine or glutamate in the forages or higher concentrations of ornithine and/or biogenic amines [e.g. GABA (from GLU), putrescine, spermidine, spermine and thermospermine (from ARG)] could be a result of plant protein degradation. This information may aid in finding an answer to the question: How are sensorially ordinary grass silages with low TP in CP a cause of dairy herd diseases?

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22.

Marginal dietary methionine concentrations and anti-oxidative status and expression of inflammatory genes in gut and liver of piglets

Marginale Methioninkonzentrationen im Futter und anti-oxidativer Status und die Expression inflammatorischer Gene in Darm und Leber beim Ferkel

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Methionine (Met) serves as a proteinogenic amino acid, but is also a precursor for molecules involved in the anti-oxidative defence system of the body, amongst them glutathione. Met supplementation above requirements has been shown to affect the anti-oxidative status of the gut (1), however, effects of Met on transcriptional activity of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2), the master regulator of the antioxidant response, are unknown. In addition, studies investigating effects of Met supplementation on the nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB), the master regulator of inflammation, are missing, but Nrf2 and NF-κB pathways are known to be interlinked (2). We hypothesized that feeding marginal dietary Met concentrations influence gene expression of Nrf2 and NF-κB target genes and the anti-oxidative status in gut and liver of piglets.

Methods: A 3-week lasting experiment was performed with 45 piglets (DanZucht x Pietrain) which were allotted to 3 groups of similar mean body weight (11.0 ± 0.9 kg). The basal diet, consisting of barley, wheat, corn starch, soybean oil, sucrose, cellulose, amino acids and minerals, was supplemented with DL-Met to reach 0.16, 0.20 and 0.24 % of dietary Met and 0.40, 0.44 and 0.48 % of dietary Met + Cysteine in groups 1, 2 and 3, respectively. Body weight and feed consumption were recorded weekly. Met concentrations were detected in plasma and liver (3). In liver and jejunum, the Trolox equivalent antioxidant capacity (TEAC), concentrations of glutathione and thiobarbituric acid reactive substances (TBARS), and the activity of the glutathione peroxidase (GPx) were determined (1). Gene expression of target genes of the NF-κB and the Nrf2 was determined by RT-PCR. The data were analysed by one-way analysis of variance considering treatment as fixed effect using Minitab.

Results: Feed intake and average daily gains increased and the feed: gain ratio decreased with increasing dietary Met concentrations. Likewise, Met concentrations increased in a dose-response relationship in plasma (8.2, 15.0 and 29.7 nmol/l for groups 1, 2 and 3; $P<0.001$) and liver (153, 179, and 246 nmol/g; $P<0.001$). In the jejunum mucosa, relative mRNA concentrations of the NF-κB target gene interleukin 8 was decreased by half in groups 2 and 3 compared to group 1 ($P=0.003$). However, gene expression of the other NF-κB target genes (TNF, ICAM) and the Nrf2 target genes (SOD1, TXNR1), and concentrations of glutathione, TEAC and TBARS, and GPx activity in the jejunum mucosa were similar between groups. In liver tissue, gene expression of the Nrf2 target gene NAD(P)H dehydrogenase, quinone 1 (NQO1), was higher in group 3 as compared to groups 1 and 2 ($P=0.004$), but that of the other Nrf2 and NF-κB target genes analysed were similar between groups. Liver concentrations of glutathione, TEAC and TBARS, and GPx activity were unaffected as well.

Conclusion: Increasing dietary Met concentrations from below requirements to requirements affected feed intake, plasma and liver Met concentrations, and growth performance in a dose-response relationship. However, gene expression of Nrf2 and NF-κB target genes as well as concentrations of lipid peroxidation products and of proteins and enzymes involved in the anti-oxidative defence system of gut and liver were mostly unaffected. This indicates that marginal dietary Met concentrations have little effect on inflammation- and oxidative stress-related pathways on the molecular level and do not change the anti-oxidative status of gut and liver in healthy piglets.

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23.

Comparison of the praecaecal digestibility of selected amino acids in alfalfa leaf mass (med. Sativa) *in vivo and in vitro*

Vergleich der praecaecalen Verdaulichkeit ausgewählter Aminosäuren in Luzerne Blattmasse (med. Sativa) in vivo und in vitro

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The aim of the study was to compare the *In vivo* digestibility of amino acids with the results of an *In vitro* method at the example of broiler chicken. Preliminary results have shown little correspondence (Sommer et al. 2015).

Method: The experiment was conducted with medium-growing broilers in the third and sixth week of life. Dried alfalfa leaves were included in the experimental diets by 10%, 30% and 50%. The diets contained TiO2 as an indigestible marker. The experimental diets were fed to 6 groups of 12 (third week of life) and/or 6 (sixth week of life) broilers, each. At the end of the testing period (i.e. 21st or 42nd day of life) broilers were killed and the chymus of the small intestine of each group was pooled and analysed for its contents of crude protein and amino acids. Subsequently, the digestibility was determined by using a linear regression model according to Rodehutscord et al. (2004). In addition, each *In vitro* digestibility of these parameters was conducted using a modified method according to Boisen & Fernandez (1997) and using the same linear regression model comparable to the *In vivo* analyzes. The same diets as in the *In vivo* trial were analyzed. In the *In vitro* method, a clearly higher number of repeats were analysed (Starter10% n=50, Starter30% n =62, Starter50% n =25; Grower10% n=50, Grower30% n =38, Grower50% n =25). The crude protein analysis and the amino acid analysis were carried out by the standard method of VDLufa. Satisfying correspondence between *In vivo* and *In vitro* was defined as the difference between the methods did not exceed 2 percent points.

Results & Discussion: Table 1 show the digestibility values for crude protein and essential amino acids of both methods. A good match between *In vitro* and *In vivo* analysis was found for Isoleucine and Leucine in the starter period, and for Methionine, Lysine, Isoleucine and Leucine in the grower period, whereas the correspondence was unsatisfying in the remaining cases. However, it has to be taken into account that the Boisen & Fernandez method (1997) made use of pepsin and pancreatin, obtained from the gastrointestinal tract of pigs as enzymes from poultry were not available. As Crevieu-Gabriel et al. (1999) emphasised that the enzyme activity is different between pig and poultry, it is assumed that this might be responsible for the differences in the digestibility of amino acids in the current investigations.

Table 1: Comparative results of the praecaecal digestibility (pcV) of crude protein and selected amino acids determined by an *In vivo* compared to an *In vitro* method in the case of broiler chicken

	Starter period		**Grower period**	
	In vivo	In vitro	In vivo	In vitro
Crude protein	92	77	88	79
Methionine	93	85	**93**	**95**
Lysine	92	98	**87**	**88**
Cystine	92	83	90	94
Leucine	88	82	**88**	**90**
Isoleucine	**85**	**84**	**87**	**88**

Conclusions: The results of the *In vitro* method provided unsatisfactory correlations with the *In vivo* method. On the other hand, the *In vitro* method offers good options to analyse a comparable high number of different feed ingredients in due time with respect to their digestibility. The results suggest a repetition of the experiment with pigs instead of broilers.

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24.

Effects of lactic acid bacteria on degradation of amino acids to biogenic amines in fermented liquid feeds

Effekte von Milchsäurebakterien auf den Abbau von Aminosäuren zu biogenen Aminen in fermentierten Flüssigfuttermitteln

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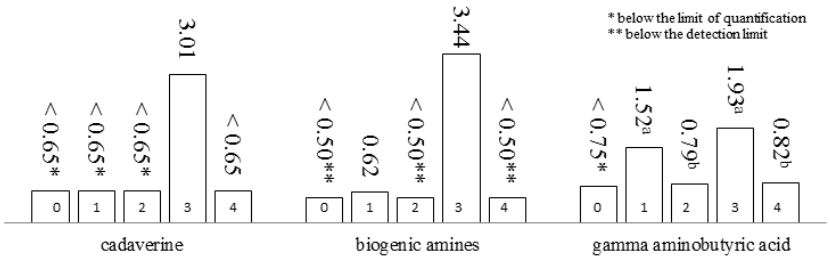
Fermentation of liquid feed by homofermentative lactic acid bacteria (LAB) is an established method to improve the storage stability and feed value. Easily soluble carbohydrates will be converted into lactic acid resulting in a fast pH reduction. Nevertheless undesired processes like decarboxylation of amino acids to biogenic amines can occur during the fermentation resulting in reduced palatability, impaired health and nutrient losses that have to be considered (1). The present study aims to investigate whether the fermentation with homofermentative LAB as feed additives can prevent the degradation of amino acids to biogenic amines and the degradation of glutamic acid to gamma amino butyric acid (GABA) in the liquid feed.

Methods: A constant quantity of barley and wheat was mixed with water (adjusted to 25 % dry matter) and inoculated with the following treatments: 1) control without additive, 2) LAB mixture (200.000 cfu/g FM → 10 mg/kg liquid feed): Three strains of homofermentative lactic acid bacteria (*Lactococcus lactis. Pediococcus pentosaceus, Lactobacillus rhamnosus),* 3) lysine HCl and 4) LAB mixture + lysine HCl. The fermentation (24h) was conducted under laboratory conditions at 37° C in 1000 ml incubators (in triplicate). The pH-value and the short chain fatty acid yield (HPLC) were recorded after 12 and 24 h. Biogenic amines and GABA (gas-phase chromatography and mass spectrometry) were analysed in basic material and treatments 1-4.Differences in GABA between treatments 1-4 were tested via ANOVA and following comparison of means (Tukey-Kramer).

Results: Fermentation (without LAB) led to a slight increase of total biogenic amines and GABA. In contrast there was no increase in the fermentation with homofermentative LAB. Furthermore, significant differences were apparent in the variants with added lysine. In contrast to the control sample the fermentation with homofermentative LAB did not increase level of cadaverine, total biogenic amines and GABA. This indicates a degradation of lysine and glutamic acid to the corresponding biogenic amines and GABA in the treatments without LAB.

Conclusion: Fermentation with homofermentative LAB can avoid the degradation of amino acids to biogenic amines. This is of particular relevance if lysine is used as additive in the diet.



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25.

Algae, mussels and ragworm as alternative protein sources in diets for rainbow trouts

Algen, Muscheln und Meeresringelwurm als alternative Eiweißquellen in Futtermitteln für Regenbogenforellen

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Aquaculture becomes more and more popular to ensure a sufficient protein supply of the increasing world population. Commercial complete diets for carnivorous fish like rainbow trouts are mainly based mainly on fish meal and byproducts originated from fish. But these ingredients, which are used for other animal species (newborns) will become more and more rare and the protein gap in agriculture production is still under discussion. This raises the question of alternative protein sources. In coastal regions marine species like algae, mussels or worms are of interest. Algae, mussels and ragworm are widely spread, currently produced in aquaculture systems or supposed to have a future production potential. Therefore, the aim of this study was to evaluate the palatability of these potential protein sources and to examine body weight gains and body composition of rainbow trouts feed with diets containing such ingredients.

Methods: A commercial complete diet for rainbow trouts was offered as control. Experimental diets were designed on the basis of this feed, supplemented with 10% red algae (A; *Delesseria sanguinea*) or 10% Baltic blue mussel (M; *Mytilus spp.*), 35% king ragworm (W; *Alitta virens*) or 10% algae and 30% ragworm (A+W). Fish meal and linseed oil were added to ensure an isonitrogenous and isocaloric (GE: 25 MJ*kg-1, crude protein: 45% i. DM) composition of the diets. The diets were fed to *Oncorhynchus mykiss* (local selection line Born LFA-MV) kept in special brackish (3 - 5 PSU) water basins. Young fish (n = 165) were randomly selected, individually marked and divided into 15 groups (three groups per diet, n = 11 fishes per basin). The experiment lasted 75 days with a feed offer of 2% of body weight per day within this time. On day 76, the fish were slaughtered and the body weight and fish composition (proportion of visceral fat and carcass) were determined. Statistics were conducted by one way ANOVA and post hoc Bonferroni t-test, performed with SigmaPlot 11.0.

Results: Feeding worms or mussels as alternative protein sources in a mixed feed for rainbow trouts resulted in a feed conversion rate that corresponds to a commercial complete diet. Tab.: Zootechnical parameters in rainbow trouts fed diets containing algae, mussels and ragworm

	C	A	W	A+W	M
proportion of the diet (%)		10	35	10 / 30	10
feed conversion rate (FCR)1	$1.17^{ab} \pm 0.08$	$1.28^{b} \pm 0.09$	$1.08^{a} \pm 0.03$	$1.17^{ab} \pm 0.07$	$1.19^{ab} \pm 0.03$
body mass - start (g)	81.6 ± 11.6	74.5 ± 12	78.5 ± 9.85	76.5 ± 8.5	77.7 ± 8.94
- end (g)	233 ± 45.7	180 ± 44.9	209 ± 42.8	200 ± 27.9	198 ± 47.9
weigth gain (%)	186	141	165	162	154
fish composition2					
- visceral fat3 (%)	2.4	2.9	2.4	2.8	2.8
- carcass4 (%)	66.3	62.9	65.5	62.7	66.2

[1] n = 3 (3 basins with n=11 fishes each) per diet [2]at day 75 [3] of the total body [4]without head

Means in the same row with different superscripts differ significantly between diets ($P<0.05$).

The addition of red algae (A) led to the significant highest FCR. Diets with mussels, ragworm or a combination of algae and ringworm led to a similar FCR compared to the control. Levels of visceral fat showed no differences between groups and indicated no negative effects of the added mussels, algae and ringworms.

Conclusion: In total, the tested marine species represent possible alternative protein sources in diets for fishes with a sufficient palatability. Especially the relative high ragworm supplementation in diet W and the lower substitution with fishmeal at the same time without notable increases in feed conversion rate might indicate for a potential use of this ingredient as a alternative protein source. Moreover, even if high amounts of these species can be found in the sea, technological methods have to be established to ensure the availability of adequate amounts of these protein sources as feed ingredients.

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26.

Selenium speciation in paired serum and cerebrospinal fluid samples of sheep

Selen-Speziation in gepaarten Proben von Serum und Liquor cerebrospinalis bei Schafen

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Selenium (Se) and selenoproteins are known to play an important role in brain function (1), however, little is known about the mode of exchange of Se or selenoproteins between the extracellular compartments blood and cerebrospinal fluid (CSF). The study presented here (2) was part of a comprehensive project on metabolic effects of differentiated Se supplementation published previously (3). It was performed to characterise total Se and Se species in CSF and serum of sheep and its relation to the respective Se concentrations in serum.

Methods: Five sheep were fed a diet with a marginal Se concentration of 0.05 mg Se/kg diet dry matter (dm, Se(-)), and five animals were fed the same diet supplemented with sodium selenite revealing a concentration of 0.2 mg Se/kg diet dm (Se(+)). All other nutrients were offered according to the recommendations for sheep. The feeding strategy was conducted for two years; At the end of the feeding period, paired samples of serum and CSF were collected and analysed using ion exchange chromatography inductively coupled plasma - dynamic reaction cell - mass spectrometry (IEC-ICP-DRC-MS) technique for total Se concentration and concentrations of Se species. Albumin concentrations were analysed additionally.

Results: There were significant differences ($p<0.01$) in total serum Se concentrations with 33.1 ± 5.11µg Se/l in the Se(-) group and 96.5 ± 18.3 µg Se/l in the Se(+) group, respectively. The corresponding total Se concentrations in CSF were 4.38 ± 1.02 µg Se/l and 6.13 ± 1.64 µg Se/l in the Se(-) and the Se(+) group ($p=0.077$), respectively. IEC-ICP-DRC-MS technique was able to differentiate the Se species selenoprotein P-bound Se (SePP), selenomethionine, glutathione peroxidase-bound Se (Se-GPx), selenocystine, thioredoxin reductase-bound Se, ovine serum albumin-bound Se (Se-OSA), SeIV and SeVI in ovine serum and CSF. Quantitatively, SePP was the main Se species in ovine serum followed by Se-GPx, both Se species were positively correlated to the total Se concentration in serum. The CSF/serum ratio (Q) of albumin (Q albumin*1000) was 6.13 ± 1.62 and 6.95 ± 1.64 for the Se(-) and the Se(+) group, respectively. The Q Se species were higher than Q albumin in both feeding groups. Significant positive regression lines ($p<0.05$) calculated for parameters in CSF vs. serum regarding all animals were found for albumin and Se-OSA only.

Conclusions The modification of the dietary Se supplementation leads to a distinctly higher total Se concentration in serum in sufficiently supplemented compared to marginal Se supplemented sheep, whereas the total Se concentrations in CSF did not reflect the nutritional management. Quantitatively, SePP is the main Se species in ovine serum followed by Se-GPx. The positive correlation to the total Se concentration attracts especially SePP to be used as a diagnostic parameter. The Q albumin reflected a physiological function of the CSF-blood barrier. Q Se species were higher than Q albumin in both feeding groups in general, supporting the hypothesis of local production of selenospecies in the brain. The positive regression line found for albumin and Se-OSA in serum vs. CSF recommends further evaluation if albumin may play a main role to convey Se across the blood-CSF barrier, prospectively. The ovine model used here, together with a highly sophisticated analytical method (IEC-ICP-DRC-MS) to characterise the Se species at both sides of the functional blood - CSF barrier, might be a worthwhile model for further studies as repeated sample collection as well as modifications of the nutritional status are feasible and effective.

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27.

Fetal programming of piglets induced by differential iron supply to pregnant sows

Fötale Prägung von Ferkeln bei unterschiedlicher Eisenversorgung tragender Sauen

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Iron deficiency anemia is a common problem in suckling piglets. High demand of 7-8 mg iron per day together with low iron stores at birth and insufficient iron supply via sow's milk induces severe deficiency symptoms during the first weeks *postpartum*. Efforts to increase iron dowry of piglets *in utero* by high iron supply of the sow during pregnancy failed due to iron homeostatic regulation at the intestinal and placental barriers. An innovative approach might be the improvement of iron utilization in newborn piglets by modulating their fetal programming via epigenetic mechanisms. Ravelli et al. (1) showed the influence of nutrient deficiency in women during pregnancy on the prevalence of obesity and diabetes type II in their offspring. In this context, the present study was designed to evaluate the epigenetic potential of iron supply of gravid sows on their offsprings.

Methods: From insemination, 20 sows were divided into two experimental groups receiving a common diet differing in iron content (114 ppm (GfE recommendation) vs. 261 ppm) for the whole pregnancy. Blood iron status parameters (hemoglobin (hb, hemocyanin method), hematocrit (hct, hematocrit zentrifugation), serum iron (photometrically), total iron binding capacity (tibc, photometrically)) of the sows were determined at the beginning and the end of pregnancy. Three piglets of 17 litters were euthanized at day 1, 5 and 21 days after birth respectively. In piglets, blood iron status parameters were measured as well as liver iron content (AAS).

Results: Initial piglet weights didn't differ between the two feeding groups (table 1), but high iron piglets showed better growth performance. In contrast to low iron group, these animals were born with higher hb and hct contents, however the values decreased in this group more drastically than in the low iron group until day 21. Serum iron contents were equal at birth in both groups (table 2). These values increased significantly in the high iron group until day 5, which is consistent with an ongoing mobilization of iron stores from the liver.

Table 1: weight, hemoglobin content and hematocrit value of piglets at days 1, 5 and 21 of life

		sow diet			
	day	**114**	**261**	**SEM**	**p- value**
weight (kg)	1	1.40	1.42	0.07	0.8868
	5	1.79[a]	2.29[b]	0.08	0.0002
	21	5.33[a]	6.31[b]	0.33	0.0180
hb (g/l)	1	76.46	86.95	3.53	0.0918
	5	74.48	68.72	3.62	0.1803
	21	72.43	61.80	6.6	0.2916
hct (%)	1	25.38[a]	31.92[b]	1.19	0.0007
	5	24.31	25.13	1.31	0.6941
	21	22.58	24.10	1.94	0.6025

Table 2: serum iron content, total iron binding capacity and liver iron content at days 1, 5 and 21 of life

		sow diet			
	day	**114**	**261**	**SEM**	**p- value**
serum iron (µd/dL)	1	39.19	40.67	0.82	0.9833
	5	45.21	85.57	0.62	0.1649
	21	18.93	18.89	0.35	0.8910
tibc (µg/dl)	1	146.88	117.23	17.62	0.1458
	5	346.47	307.06	30.50	0.2669
	21	658.51	618.00	81.08	0.6421
liver iron (mg/kg)	1	499.70	808.90	175.12	0.0926
	5	253.50	201.27	64.893	0.8330
	21	94.12	101.42	16.278	0.3990

SEM = standard error of means; different superscripts indicate significantly different treatment means

Conclusion: Higher iron stores in liver at birth and improved growth of piglets in high iron group indicate that the current iron supply recommendations of sows in pregnancy are insufficient to fill piglets iron stores *in utero*. Moreover, this study shows that the positive effect of a higher iron dowry is only temporarily and not persistent. Rather, this study suggests that offspring of inadequately supplied sows possibly develops an iron thrifty phenotype reflected by compensatory growth and more constant preservation of iron status parameters in the blood. Further analyses on transcriptomic and epigenetic levels are still in process.

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28.

Skeleton health in growing pigs - Investigations on the inter-/intraindividual variation of different bone parameters in pigs of different ages

Skelettgesundheit bei wachsenden Schweinen - Untersuchungen zur inter-/intraindividuellen Variation von verschiedenen Knochenparametern bei Schweinen verschiedener Altersgruppen

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Introduction: Many studies have been made to investigate the effects of mineral deficiency on bone integrity but the question which type of bone or which parts of different bones to select for diagnosis of insufficient mineralisation at different ages is rarely answered (1). Among other aspects the "ideal" parameter should only vary slightly in healthy pigs. Therefore the individual variation of different bone parameters in several bones should be taken into account when selecting a distinct bone for diagnostic purposes. Thus the aim of this study was to investigate the physiological development of several physical and chemical bone parameters in different bones from healthy growing pigs (healthy: defined by clinical and pathological investigations).

Methods: Nine weaned pigs were fed a conventional fattening diet and slaughtered at different ages (12, 16 and 24 respectively 28 weeks; mean body weight: 35.7 kg ± 1.53, 54.0 kg ± 3.04 and 101 kg ± 14.1). The humerus and the third metacarpal bone (MC III) were measured (length, diameter, cortical thickness), weighed and subsequently the proximal part of the humerus and the third metacarpal bone (in toto) were analysed for their density (Archimedes´ principle) and chemical composition (dried by lyophilisation, extracted in petroleum ether and ashed at 600°C; Ca and P were analysed by atomic absorption resp. colorimetrically after microwave digestion).

Results: The different bones proportionally showed similar growth in length and development in density (Hum: 1.13 → 1.14 g/cm^3; MC III: 1.15 → 1.22 g/cm^3) but development of cortical thickness differed between humerus and MC III during the two periods described. Thus the increase in cortical thickness in MC III was higher from wk. 12 to wk. 16 (factor: 1.28) than from wk. 16 to wk. 24 resp. 28 (factor: 1.03). In contrast the cortex of humerus gained relatively more thickness between wk. 16 and wk. 24 resp. 28 (factor: 1.22 vs. factor: 1.07 from wk. 12 to wk. 16). The chemical compositions of both bones of pigs of different ages are shown in table 1.

Tab. 1: Chemical composition of the proximal humerus epiphysis and of os metacarpale III (mean value ± standard deviation)

age	ff DM1 [g/kg FW]		ash [g/kg ff DM]		Ca [g/kg ff DM]		P [g/kg ff DM]	
[weeks]	Hum	MC III	Hum	MC III	Hum	MC III	Hum	MC III
12	339 ± 15.7	420 ± 25.6	401 ± 7.84	476 ± 8.60	143 ± 10.7	194 ± 6.66	64.4 ± 5.66	89.4 ± 0.26
16	325 ± 11.4	445 ± 12.7	436 ± 8.18	*	150 ± 21.2	195 ± 6.56	75.5 ± 7.00	93.7 ± 0.75
24 resp. 28	336 ± 9.65	474 ± 19.9	460 ± 19.7	555 ± 8.09	162 ± 4.75	206 ± 4.58	75.8 ± 0.50	97.5 ± 1.39

[1]**ff DM**=fat-free dry matter (contains less than 1% fat); **FW**=fresh weight; **Hum**=proximal part of the humerus, including the entire growth plate; **MC III**= Os metacarpale III; * values missing due to methodological problems

Except for the DM-content in the humerus all parameters increased with aging. In comparison to the humeri the metacarpal bones showed higher values in all parameters examined but the development by tendency was the same in both bones.

Discussion: Although there are obvious changes in physical and chemical characteristics during the aging process in both types of bones, humeri and metacarpal bones showed different properties in their processes of growth. This raises the question whether a specific bone or localisation within the bone reacts more sensitive to mineral deficiencies in periods of maximal bone formation. Furthermore it has to be considered which bones are easy to acquire from slaughter pigs without drastic impairment of carcass value. Further investigations are needed to decide which bone or part of bone is most suitable for diagnostic purposes at different ages.

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29.

Interactive effects of P, Ca and phytase supplements on $InsP_6$ degradation and net absorption of P and Ca up to the lower ileum of broiler chickens

Wechselwirkungen von P, Ca und Phytase Zulagen auf den $InsP_6$-Abbau und P- und Ca-Absorption bis zum hinteren Ileum von Broilern

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A previous study has shown a negative effect of monocalcium phosphate (MCP) addition on inositol hexakisphosphate ($InsP_6$) degradation and P net absorption in broiler chickens fed diets without or with added phytase [1]. It remained unclear whether the effects were caused by Ca, perhaps through influencing phytate solubility or by inorganic P inhibiting $InsP_6$ degradation as the end product. Therefore, the objective of this study was to distinguish between effects of P and Ca on $InsP_6$ degradation and P and Ca net absorption when added separately with or without microbial phytase.

Methods: At 15 days of age, 7 pens with 19 broiler chickens each were randomly allocated to the treatments. The treatments were arranged in a 2x2x2-factorial and included diets without (P-, 4.1 g P/kg DM) or with (P+, 6.9 g P/kg DM) monosodium phosphate supplementation, without (Ca-, 6.2 g Ca/kg DM) or with (Ca+, 10.4 g Ca/kg DM) fine limestone supplementation and without or with supplementation of 1500 FTU/kg of a modified, *E.coli* derived 6-phytase (Quantum™ Blue). At 27 days of age, digesta from a defined section of the lower ileum was collected, pooled on a pen basis and freeze dried. Samples were analyzed for total P, Ca, $InsP_6$ and titanium dioxide (TiO_2) which was used as the indigestible marker. A three-factorial ANOVA was carried out using SAS 9.3. Significance was declared at $P < 0.05$.

Results: Significant interactions were detected for all traits and are indicated by the superscript letters (Table). Average body weight (BW) and BW gain were lowest and feed:gain (F:G) highest in treatment P-Ca+ without phytase and were significantly affected by added phytase. Adding P increased performance of the broilers when no phytase was added. There were no differences between treatments P-Ca-, P+Ca- and P+Ca+ in the presence of phytase except F:G that was lowest in P+Ca+. The lowest $InsP_6$ hydrolysis was found in treatment P+Ca+ in the absence of phytase. Adding only Ca had no significant effect whereas adding P had a negative effect when phytase was not present. With phytase in the diet no significant effects of the other factors were found. P net absorption was increased by adding phytase or when mineral P was added. Ca addition had a negative effect on P net absorption. Ca net absorption was lowest in P+Ca+ with and without phytase addition. There were no differences between P-Ca- and P-Ca+ and no increase by phytase addition. Phytase increased Ca net absorption only in P+Ca-.

Table: Performance, $InsP_6$ degradation, Ca and P net absorption up to the lower ileum

Phytase level	0				1500				SEM
	P-Ca-	P-Ca+	P+Ca-	P+Ca+	P-Ca-	P-Ca+	P+Ca-	P+Ca+	
BW d 27, g	1528^{b}	1270^{c}	1625^{a}	1608^{a}	1640^{a}	1509^{b}	1646^{a}	1640^{a}	19.0
BW gain, g/d	80b	59^{c}	89^{a}	$87a^{a}$	88^{a}	79^{b}	90^{a}	90^{a}	1.2
Feed:gain (g/g)	1.50^{b}	1.67^{a}	1.44^{c}	1.43^{c}	1.42^{c}	1.47bc	1.42^{c}	1.30^{d}	0.02
InsP6 degradation, %	56^{b}	54^{b}	40^{c}	21^{d}	87^{a}	77^{a}	87^{a}	77^{a}	2.8
P net absorption, %	48^{d}	41^{e}	62^{c}	48^{d}	68^{b}	60^{c}	76^{a}	59^{c}	1.7
Ca net absorption, %	57^{ab}	55^{ab}	51^{c}	41^{d}	53^{bc}	50^{c}	57^{a}	38^{d}	1.2

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

Conclusions: Phytase addition can compensate for negative Ca effects on broiler performance. Added P reduced $InsP_6$ degradation in diets not containing phytase. Ca reduced $InsP_6$ degradation when supplemented together with P, but not alone. Phytase supplemented at the level of this study compensated for all decreasing effects of Ca and P supplements. The results suggest that Ca restriction and phytase application in broiler feed benefits P utilisation and performance.

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30.

Effect of dose and source of dietary Copper supplementation on rumen dry matter degradation in non-lactating Holstein cows

Effekt von Dosis und Quelle bei der Kupfersupplementierung auf den ruminalen Trockenmasseabbau bei trockenstehenden Holstein Kühen

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Question: Ruminant diets have to be supplemented with the essential trace mineral Copper (Cu). Otherwise, Cu is known for its antibacterial effects when applied in excess, e.g. as growth promoter in piglets. In case of ruminants, however, the impact of Cu on the microbiome is not well understood. The following study presents the effect of varying dietary Cu supplementations (dose, source) on ruminal dry matter degradability.

Methods: The study was conducted with 7 non-lactating rumen-fistulated cows according to the *in situ*-method. 6.5 kg DM of TMR based on grass silage, maize silage, wheat and soybean extracts were offered in two equal portions per head and day. Cu was added either as sulfate ($CuSO_4$) or tri-basic chloride (TBCC) in order to obtain three levels of total dietary Cu concentrations: 10 mg/kg DM (recommended supply (1)), 35 mg/kg DM (maximum according to feed law), and 50 mg/kg DM (mild excess). $CuSO_4$ and TBCC represented Cu sources with presumably high and low ruminal solubility. Every cow received each of the 6 treatment combinations along 6 experimental periods according to a split-plot design. The TMR was incubated into the rumen via nylon bags along the following schedule: 0, 1.5, 3, 6, 9, 12, 24, and 48 hours, starting before morning feeding. The observed *in situ* dry matter degradability (ISDMD) was used to estimate rate of degradation of insoluble but degradable fraction (RD).

Results: Supplementation of Cu from sulfate at mild excess stimulated rumen DM degradation between 6 and 12 h after feeding. Varying doses of Cu from TBCC, however, remained ineffective until 6 h but reduced ISDMD around 9h post feeding. Overall, rising doses of Cu from sulfate increased RD (orthogonal contrast: 10 mg/kg vs. 50 mg/kg DM, $p \leq 0.05$) while TBCC showed numerically slightly depressive effects.

Source	Dose (mg/kg)	ISDMD (%)							RD (%/h)
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h	
$CuSO_4$	10	43.4	46.2^{b}	53.2^{b}	61.7^{ab}	66.2	78.9	86.5	7.13
	35	43,4	46.9^{ab}	55.0^{b}	62.2^{ab}	66.7	78.8	85.9	7.58
	50	43.6	47.4^{ab}	58.0^{a}	64.3^{a}	68.3	78.8	86.1	8.60
TBCC	10	43.1	47.8^{a}	54.8^{b}	64.3^{a}	66.1	79.5	85.9	7.68
	35	43.1	46.8^{ab}	53.7^{b}	62.5^{ab}	68.0	79.4	85.9	7.78
	50	43.5	46.6^{ab}	55.6^{b}	60.5^{b}	66.1	79.7	86.8	7.24
	SEM	0.28	0.36	0.62	0.81	0.77	0.51	0.23	0.52
	ANOVA	0.73	0.05	0.01	0.01	0.14	0.62	0.30	--

Conclusion: The different reaction of DM degradation at high Cu doses from $CuSO_4$ (high response) vs. TBCC (small response) seems to reflect differences in ruminal solubility of these sources. Stimulation of rumen DM degradation through high doses of soluble Cu might arise from changes in the rumen microbiome that need to be further clarified. In total, mild Cu excess (50 mg/kg DM) does not seem to consistently impair rumen fermentation.

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31.

Influence of iron supply in pregnant sows on litter size and piglet weight

Einfluss der Eisenversorgung bei trächtigen Sauen auf Wurfgröße und Ferkelgewicht

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According to NRC (1) and GFE (2), recommendations for iron supply in pregnant and lactating sows is 100 mg/kg diet. This value exists for more than 35 years and was evaluated for the last time in 1985 (3). However, within the last decades reproductive performance of sows has increased significantly by breeding progress making it questionable, if recommendations are still valid. In this study the influence of differential iron supply to sows during gestation on reproductive performance should be assessed.

Methods: 20 multiparous sows were divided into two groups with equal average iron state and were supplied with common diets from insemination to farrowing. The diets differed significantly in iron supplementation. Iron content in the low iron group was according to actual recommendations (114 mg/kg DM) and high iron group was additionally supplemented with 147 mg $FeSO_4$/kg diet to a total iron content of 261 mg/kg DM. After farrowing, litter size, number of piglets born alive and piglet weights were recorded. Data were analyzed with two-way ANOVA (sow, diet) using the GLM procedure in the SAS 9.3 software package. Significantly different means were tested with Student-Newman-Keuls method at the 5% significance level.

Results: Litter size was significantly different between the two feeding groups (Table 1). In average, sows from the low iron group received 3 piglets less than sows fed with high iron diet. Concomitantly, number of piglets born alive was decreased by 29% in low iron group compared to high iron group. Mean piglet weights showed no differences between experimental groups, but total litter weights were significantly higher in high iron group.

Table1: Litter size, piglets born alive, piglet weight and litter weight of newborn piglets

	sow diet			
	114 ppm	261 ppm	SEM	p-value
litter size	9.30[a]	12.30[b]	2.20	0.0220
piglets born alive	8.50[a]	11.40[b]	2.30	0.0238
percentage of living piglets	92.85	91.08	10.68	0.6921
piglet weight (kg)	1.51	1.57	0.13	0.5376
litter weight (kg)	14.00[a]	19.30[b]	2.70	0.0241

SEM = standard error of means; different superscripts indicate significantly different treatment means

Conclusion: In this study both feeding groups were supplied with adequate iron contents according to current recommendations of NRC and GFE. Nevertheless, a massive decline in reproductive performance could be demonstrated in low iron group confirming the assumption of an increased iron demand during pregnancy due to improved performances following breeding processes. Subsequently, iron requirements of pregnant and lactating sows have to be reevaluated and, in consequence, recommendations have to be adjusted for modern pig lines.

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32.

Zip4-like zinc transporter variability is widespread in pig breeds but not in Göttinger Minipigs and should be considered in experimental Zinc metabolic studies

Der Zip4-like Zinktransporter zeigt genetisch bedingte Variation in Schweinerassen jedoch nicht beim Göttinger Minipig und sollte bei Zink Stoffwechselstudien Berücksichtigung finden

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Dietary zinc (Zn) can influence growth and health of weaning piglets. On the other hand Zn can cause environmental problems if its emission via manure is too high. An A>C-SNP within exon 9 (p.Glu477Ala) of porcine zip4-like zinc transporter gene (*Zip4-like*) has recently been demonstrated in a small number of pigs. The SNP showed significant associations to pancreatic Zn concentration and apparent Zn absorption of piglets, whereas allele A seems to be the advantageous allele (1). Therefore it is important to know the occurrence of this SNP in different pig population including minipigs especially as basis for Zinc metabolic studies.

Methods: 242 samples (tails, testes, semen) from different breeds were collected according to the German Animal Welfare Act. DNA-isolation was performed with a commercial DNA extraction kit. In addition DNA samples from wild pigs (n=41) sampled in different regions of Germany, Göttinger Minipigs (n=37) and from former studies (n= 84) were available (2). For genotyping of the A>C- SNP within exon 9 of the porcine Zip-4 like gene (Ensembl No. rs80803395) a PCR based screening method was developed.

Results: Total agreement between the established DNA based SSCP test and the sequencing results was observed. A high variability could be demonstrated in all breeds including wild pig but not in Göttinger Minipigs (Table 1). In all breeds except Hampshire allele C is predominant.

Table 1 Allele and genotype frequencies of the A>C- SNP within exon 9 of the porcine Zip-4 like gene in different pig breeds including wild pigs and Göttinger Minipigs

		Allele frequencies		Genotype frequencies		
Breed /Population	n	A	C	AA	AC	CC
Dt. Landrasse	52	0.163	0.837	0.019	0.288	0.693
Dt. Edelschwein	18	0.222	0.778	0.000	0.444	0.556
Schwäbisch Hällisches Schwein	20	0.425	0.575	0.100	0.650	0.250
Hampshire	27	0.537	0.463	0.333	0.407	0.260
Pietrain	144	0.271	0.729	0.042	0.458	0.500
Large White	16	0.281	0.719	0.063	0.438	0.499
Duroc	21	0.167	0.833	0.048	0.238	0.714
White Duroc	28	0.464	0.535	0.286	0.357	0.357
Wildschwein	41	0.390	0.610	0.171	0.439	0.390
Göttinger Minipig	37	0.000	1.000	-	-	1.000

Conclusion: The SNP in the_Zip4-like zinc transporter gene is common in all breeds and indicates that genotyping especially of experimental animals can be a powerful tool to reduce the inter individual variance in experiments dealing with Zn absorption. In addition as the genetic variation is associated with efficiency of Zn absorption (1) the study offers new possibilities in the field of piglets nutrition.

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33.

Effect of varying phosphorus levels in diets of weaned piglets on the bacterial microbiome in colon and faeces

Effekt einer unterschiedlichen Phosphorversorgung auf das bakterielle Mikrobiom in Colon und Kot bei Absetzferkeln

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Phosphorus is a major element with potential impact on the intestinal microbiota of pigs. Studies revealed that bacterial growth is limited in the intestine when low concentrations of phosphorus are fed (1) and that ileal bacteria increased with higher calcium and phosphorus feed contents, whereas there was no effect on bacterial numbers in the colon (2). Due to a reduced content of faecal acetic acid and ammonia as well as a lower incidence of diarrhea observed in phosphorus undersupplied piglets in a previous study (3), it was assumed that phosphorus affects the intestinal health status and microbiome. Therefore, the aim of this study was to evaluate the effect of varying phosphorus levels in diets of piglets on the bacterial microbiome of colon and faeces.

Methods: A repeated trial with 18 weaned German Landrace piglets was conducted. Animals were divided into 3 groups (n = 6) fed a pelleted diet (on the basis of wheat, barley and soybean; supplied with varying levels of monocalcium phosphate) with soluble phosphorus contents of 0.32% (Low-P), 0.54% (Normal-P) and 0.74% (High-P) of dry matter. On day 35 (d35), samples were taken from each piglet before slaughtering (faeces) and after slaughtering (intestinal chyme). Colonic and faecal samples of 3 piglets were randomly selected in each group for molecular biological analysis. Bacterial DNA was isolated and amplified *via* polymerase chain reaction. Afterwards, a denaturing gradient gel electrophoresis (DGGE) was performed and sequencing was conducted by Eurofins (Ebersberg, Germany). Sequences were compared with the database of the National Center for Biotechnology Information using the Basic Local Alignment Search Tool. The DGGE band patterns were analyzed with Bionumerics 5.0 (Applied Maths, Inc., Sint-Martens-Latem, Belgium).

Results: Dietary P-level neither affected DGGE band patterns nor the appearance of species in the different samples. Less than 60% similarities between band patterns of repeated trials were found, whereby band patterns of colonic and faecal samples of each experiment showed similarities of up to 80%. Dominating species in colonic and faecal samples of both trials were *Roseburia faecis*, *Eubacterium cellulosolvens*, *Sarcina ventriculi* and *Butyrivibrio fibrisolvens,* which are involved in the bacterial degradation of indigestive polysaccharides (except *Sarcina ventriculi*). Facultative pathogens (e. g. *Streptococcus gallolyticus*) were rarely detected and could not be identified in the Low-P group. *Lactobacillus* spp. was documented in colonic and faecal samples of solely one piglet.

Conclusion: Using DGGE, no clear effect of the dietary P-level on the colonic and faecal bacterial microbiome was detected and there was no apparent effect of the diets on the occurrence of species or DGGE band patterns. Further experiments using quantitative methods and additional intestinal sections as well as feeding trials are necessary for final conclusions. Furthermore, the potential impact on metabolic processes as well as the effect on fungi and protozoa should be investigated. Assuming that there is no significant impact on the intestinal microbiome, a P-reduction in swine nutrition might be feasible without negative effects on the intestinal health.

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34.

Opportunities to reduce the sampling frequency for measurement of duodenal dry matter flow and ruminal microbial crude protein synthesis in dairy cows

Möglichkeiten zur Reduzierung der Probeentnahmefrequenz zur Messung des duodenalen Trockenmasseflusses und der ruminalen mikrobiellen Rohproteinsynthese bei Milchkühen

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Measurement of duodenal dry matter flow (DMF) and estimation of duodenal microbial crude protein flow (MCP) according to techniques developed in Braunschweig (1) requires intensive sampling of duodenal chyme. The objective of the present study was to investigate opportunities to reduce intensive sampling without loss in accuracy.

Methods: Seven pluriparous dry German Holstein cows fitted with rumen and duodenum cannulae were housed in a tie stall barn. Cows were fed a diet consisting of 50% grass silage and 50% maize silage (on dry matter basis) for *ad libitum* intake. The experiment lasted 19 days. The first 14 days were allowed for equilibration to the experimental diet and the remaining 5 days were the sampling period. Samples of duodenal chyme were taken every two hours (h) during the five consecutive days of sampling. At each sampling 600 mL duodenal chyme were collected. Aliquots of 100 and 400 mL were generated. The 100 mL aliquot was pooled on each day over 24 h and represented a 2 h sampling frequency (2 h FRQ). The 400 mL aliquots obtained every 10 and 12 h represented a 10 h (10 h FRQ) and a 12 h (12 h FRQ) sampling frequency. The pooled samples of the 2 h FRQ and each 400 ml aliquot from the 10 h FRQ and 12 h FRQ were analyzed. For calculation of the daily DMF Cr_2O_3 was used as a marker. The microbial N fraction of the duodenal non-ammonia N was estimated by NIRS (2) to calculate daily MCP. Correlation coefficients were calculated and ANOVA was conducted by using STATISTICA software version 12.

Results: For DMF the highest correlation coefficient (r = 0.94) was observed for the 2 h FRQ with the 10 h FRQ. The regression coefficient for linear regression of the 2 h FRQ on the 10 h FRQ was closest to 1 and the intersection of the ordinate was closest to zero (Figure 1). The correlations of MCP estimated from 2 h FRQ sampling with the estimated values from the 10 h FRQ and 12 h FRQ sampling were equal. The regression coefficient and intersection of the ordinate suggested a higher accuracy of the 10 h FRQ for MCP (Figure 2). Overall, significant effects of sampling FRQ on DMF (*P* = 0.626) and MCP (P = 0.999) were not observed.

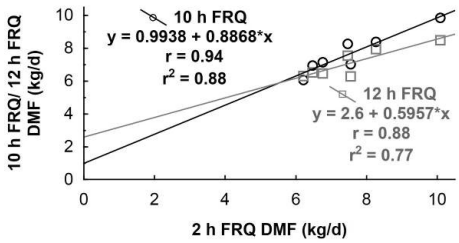

Figure 1: Relationship of duodenal dry matter flows (DMF) calculated based on 2 h sampling FRQ with 10 h or 12 h FRQ.

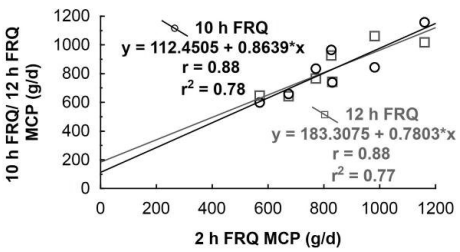

Figure 2: Relationship of duodenal microbial crude protein flows (MCP) calculated based on 2 h sampling FRQ with 10 h or 12 h FRQ.

Conclusion: The reduction of the sampling frequency of duodenal chyme from every 2 h to every 10 or 12 h over 5 days seems applicable. Related to the 2 h sampling FRQ the 10 h sampling FRQ showed the highest accuracy of estimation for DMF and MCP.

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35.

Balance studies on dietary intake and excretion pathways of glyphosate in lactating dairy cows

Bilanzierende Studien zu Aufnahme und Ausscheidung von Glyphosat bei laktierenden Kühen

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Glyphosate (*N*-phosphonomethylglycine) is a broad-spectrum, non-selective herbicide and the most used agent in agriculture worldwide for weed control and plant growth regulation. Its mode of action consists of an inhibition in synthesis of aromatic amino acids. In 2012, the annual global glyphosate (**GLY**) production volume was 720.000 tons and approximately 45% of the global production volume was administered to glyphosate-resistant transgenic plants (i.e. soybean, corn, cotton, canola). In comparison with other herbicides it was generally assumed that **GLY** is characterized by a low toxicity associated with a high environmental sustainability. However, it has been discussed in recent years that glyphosate might affect rumen microbial metabolism and diversity. In addition, its potential role as a food contaminant by excretion across the mammary gland has also been taken into consideration. Since there are no data on dietary intake and the excretion pathways in dairy cows it was the aim of the present study to quantify intake, fecal, renal and milk excretion of **GLY** as its major degradation product in lactating dairy cows.

Methods: Samples originated from six balance experiments with an overall number of 32 lactating dairy cows of the German Holstein breed. All animals were equipped with a rumen and a duodenal cannula. In each balance experiment between 4 and 6 cows were used and measurements on feed intake and total collection of feces, urine and milk were performed in the present study. The diets were based on corn silage and concentrates in different proportions. In two experiments duodenal contents could also be collected. The samples were analyzed for **GLY** by applying LC-MS/MS (milk, feed) or GC-MS/MS (feces, urine, duodenal contents).

Results: The GLY concentrations in concentrates ranged between 0.02 and 0.72 mg/kg dry matter. In only one balance experiment GLY could also be detected in corn silage (0.035 mg/kg dry matter). Corn silage in all other experiments contained GLY lower than the limit of quantification. In all balance experiments dietary GLY intake ranged between 0.08 and 6.67 mg/day. Irrespective of daily intake 61% of intake was excreted with feces whereas 8% of GLY intake was excreted with urine. In all milk samples GLY concentrations were below the level of quantification. From both experiments with collection of duodenal contents the flow of GLY into the proximal duodenum was lower than dietary intake and was equivalent to 94 and 64% of intake.

Conclusion: The present data represent the first data on quantitative aspects of intake and excretion of GLY in lactating dairy cows under conventional feeding systems. From these data it can be concluded that the gastrointestinal availability of GLY is low in dairy cows and that at intake levels below 7 mg/day the excretion pathway across the mammary gland has no relevance. At present, there is no conclusive evidence on the proportion of GLY which is not excreted via feces and urine. Either a potential ruminal degradation or a gastrointestinal absorption have to be taken into account and should be studied in further experiments.

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36.

Metabolic adaptation in transition dairy cows experiencing different lipolysis early postpartum

Metabolische Adaptation von Kühen in der Transitphase mit unterschiedlicher Fettmobilisierung

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Introduction: Metabolic adaptation includes an array of concerted metabolic and endocrine events that enable dairy cows to bridge the gap of the energy deficit at the onset of lactation (1). Newer research suggests a large variation among dairy cows in the release of energy from stored adipose tissues (2). The aim of this study was to evaluate individual differences in metabolic and endocrine adaptation in early-lactating dairy cows experiencing moderate and various energy deficits and fat mobilization postpartum.

Methods: A total of 30 (12 primiparous and 18 multiparous) pregnant cows were fed the same close-up and fresh lactation diets and kept in the same management conditions. Blood samples were collected at d -14, and -4, relative to expected parturition, and at d 2, and 21 postpartum. Serum metabolites and hormones related to glucose and lipid metabolism, as well as concentrations of several liver enzymes and acute phase proteins were determined. Body weights and milk yields were recorded and balances of NE_L and utilizable protein at the duodenum (uCP) were assessed on a weekly basis by comparing the individually measured intake in NEL and uCP to the corresponding individual demands for pregnancy and growth, milk production and estimated losses (3). Based on serum concentration of non-esterified fatty acids (NEFA) postpartum, cows were retrospectively classified into low (LOW, n = 8), medium (MEDIUM, n = 11), and high (HIGH, n = 11) fat-mobilization groups, with NEFA levels of < 0.4 mmol/L, between 0.4 and 0.7 mmol/L, and > 0.7 mmol/L, respectively. Statistical analysis was performed using the MIXED procedure of SAS, including the fixed effects of day or wk relative to calving, parity and mobilization as well as the resulting 2-way interaction between day or wk relative to calving and mobilization.

Results: Overall, elevated NEFA concentrations in the HIGH group went along with a higher ratio of NEFA to cholesterol ($P < 0.01$), tendentially higher insulin concentrations ($P = 0.08$) as well as reduced insulin sensitivity as indicated by the revised quantitative insulin sensitivity check index (RQUICKI; $P < 0.01$). While serum glucose, energy deficit and BW loss did not differ, cows of the HIGH group exhibited a trend towards an increased lactate concentration in the serum, compared to the MEDIUM group ($P = 0.06$). No differences in liver enzymes were evidenced among mobilization groups, whereas concentrations of bilirubin showed lowest concentrations in the LOW group ($P < 0.01$). Furthermore, a trend towards an interaction between day relative to calving and mobilization for haptoglobin revealed lowest concentrations after parturition in group LOW ($P=0.07$). Data of milk yield and milk energy output showed no significant differences among cows of different fat mobilization degrees, despite changes in energy ($P = 0.02$) and uCP balance ($P = 0.02$) as well as BW change postpartum ($P = 0.05$). Tendentially higher DMI was observed in cows of the LOW group ($P = 0.07$).

Conclusion: The study revealed similarities but also differences in metabolic and endocrine adaptation strategies in early-lactation cows. Although all cows had similar levels of blood glucose and milk production, they showed differences in levels of circulating NEFA, differed in insulin sensitivity and concentration of lactate. Different NEFA levels did not reflect similarities in deficits of NEL and uCP in cows with medium and highest lipolysis levels, but mirrored the lower deficits in the LOW group. The latter cows seem to regulate their homeorhesis by tending to increase DMI which alleviated the energy deficit and prevented strong body weight losses.

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37.

Methane production of five non-ruminating foregut fermenting mammals

Methanproduktion von fünf nicht-wiederkäuenden Vormagen-fermentierenden Säugern

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Methane (CH_4) production varies between herbivore species, but reasons for this variation remain to be elucidated. Ruminants typically produce more methane than other herbivores (1). Because ruminants are foregut fermenters, this raises the question whether it is foregut fermentation *per se* or rumination that causes these high emissions. Based on limited measurements in a kangaroo species that had CH_4 emissions in the range of similar-sized hindgut fermenters, it had been suggested that the difference is rather linked to the presence or absence of rumination than the presence or absence of a forestomach (2). To further investigate this question, we measured CH_4 production in five species of non-ruminant mammalian herbivores with complex forestomachs - a sloth, a peccary, a hippopotamus and two kangaroo species -, and compared the results to previously published data on domestic and wild herbivores.

Methods: Methane production was measured by open-chamber respirometry in four Linnè's two-toed sloths (*Choloepus didactylus* mean body mass 10 kg), six western grey kangaroos (*Macropus fuliginosus,* 22 kg), four red kangaroos (*Macropus rufus,* 18 kg), four collared peccaries (*Pecari tajacu*, 17 kg) and four pygmy hippopotamus (*Hexaprotodon liberiensis*, 229 kg). Except for sloths (which received their usual zoo diet composed of fruits and vegetables, and which typically do not accept other diets), animals were fed lucerne-based diets provided at ad libitum access. Exclusively in kangaroos, a second food intake level was tested meeting only 75 % of maintenance energy requirements. Food intake and digestibility were measured in all species as well. Additionally, mean digesta retention times were measured in sloths, peccaries and pygmy hippos. Results were combined with published data for sheep and ponies to investigate correlations between CH_4 yield, intake and retention times, and compared to other published data on domestic ruminants, hindgut fermenters, and pigs. Statistics included correlation analyses and General Linear Models using data of all species, and ANOVA with intake level and species for the kangaroo species alone.

Results: Average mean digesta retention times ranged from 41 h in peccaries to more than 140 h in sloths. Methane production averaged as follows: Linnè's two-toed sloths: 3 L/d, 33 L/kg dry matter intake (DMI), 7.7 % of gross energy intake (GEI); western grey kangaroos red kangaroos: 3 L/d, 8 L/kg DMI, 1.6 % GEI for each species separately; collared peccaries: 8 L/d, 18 L/kg DMI, 4.0 % GEI; pygmy hippopotamus: 72 L/d, 19 L/kg DMI, 4.2 % GEI. Within kangaroos, CH_4 yield (per dry matter intake) was higher on the reduced food intake level, approaching levels previously reported in ruminants, and leading to similar absolute daily emissions on both intake levels. Across the other species, CH_4 yield (per dry matter intake) was also negatively correlated to food intake, and positively correlated to mean digesta retention times.

Conclusion: The measurements in kangaroos corroborated previous findings of comparatively low absolute daily methane emissions in this herbivore group (3). From the present findings, it is evident that the distinction on whether a species is a foregut fermenter or not, or whether it ruminates or not, is not sufficient to explain the variation in CH_4 production between species. Rather, differences in CH_4 production between species on similar diets appear related to species-specific differences in food intake levels and digesta retention kinetics.

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38.

Effect of taurine supplementation on fat (ether extract) digestibility in pigs with experimentally induced pancreatic exocrine insufficiency

Einfluss einer Taurinergänzung auf die Fettverdaulichkeit bei Schweinen mit experimentell induzierter exokriner Pankreasinsuffizienz

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Exocrine pancreatic insufficiency (EPI) is a common disease in human and veterinary medicine. The maldigestion and malabsorption of ether extract (EE) resulting in steathorrea is the most relevant symptom [1]. Pancreatic enzyme replacement therapy (PERT) is the established treatment but even by use of modern enzyme products the EE digestibility (EE dig.) often cannot be normalized [2]. As EPI causes a reduced reabsorption of bile and furthermore reduced serum levels of taurine were observed in juvenile pigs with EPI [3] it was hypothesized that taurine deficiency might force malabsorption of EE and that oral taurine supplementation might be beneficial for improvement of EE dig. in case of EPI treated with PERT. The aim of this study was to check, whether oral taurine supplementation can improve EE dig. in pigs with experimentally induced EPI - used as a model for humans.

Material and Methods: The study was performed on 7 adult female Göttinger minipigs (Ellegaard) in which an EPI was experimentally induced by ligation of the pancreatic duct. The animals were fed a complete high-fat diet (containing 285 g EE / kg dm) twice a day (233 g dm per meal). The study was divided into two parts: in study A the animals received the high fat diet with a multienzyme-product (Creon®, Abbott Laboratories GmbH, Neustadt, Germany) accounting for 100.000 I.U. lipase per meal; in study B diet and enzyme dosage were identical but 1g taurine (myprotein, Northwich, UK) was added to each meal. The EE dig. was determined during a 5 days lasting faeces collection period following a 10-days adaptation period. Chromium oxide (Cr_2O_3, Sigma Aldrich Chemie) added to the diet in a dosage of 2.5 g / kg was used as a marker. Statistical analysis was performed using UNIVARIATE procedure; signed rank test (SAS)

Results: The addition of taurine caused a significant ($p<0.05$) increase of EE dig.: Pigs with experimentally induced EPI receiving 100.000 I.U. lipase per meal showed a mean EE dig. of 65.4 ± 6.99 %. When 1 g taurine was added the mean EE dig. reached 69.7 ± 5.45 %. Interestingly the effect of adding taurine differed markedly between the animals: In 6 out of 7 animals there was an increase of EE dig. varying from 2.69 up to 9.34 %. In one animal (number 4) there was no positive effect of taurine supplementation (see figure 1).

Figure 1: Ether extract digestibility in pancreatic duct ligated pigs fed a high fat diet and 100.000 I.U. lipase per meal with or without 1 g of taurine per meal

Discussion: The moderate lipase dosage used in this study (1515 I.U. per g fat) was chosen to make it easier to detect changes caused by taurine supplementation. The addition of taurine to the diet (1g) caused a significant increase of EE digestibility. As there are no negative side effects and taurine supplementation can be regarded as being safe, the use of taurine is recommendable in any case if EE digestibility cannot be treated satisfactory by use of PERT. Nonetheless the high variation in individual response is noteworthy and should be taken into account. The individual taurine status of the animals might be relevant for this variation but was not checked in this study.

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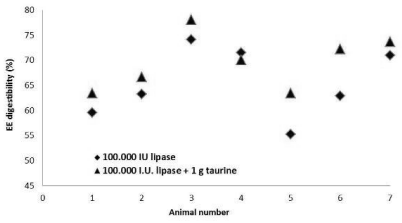

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39.

Apelin and its receptor are expressed in the mandibular gland of growing pigs: influences of different physical forms of the diet on their presence and localization

Apelin und Apelin-Rezeptor - ihr Vorkommen in der Speicheldrüse (Gl. mandibularis) junger Schweine unter dem Einfluss der Futterstruktur

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The apelinergic system is a complex system including the apelin peptide (AP) and its related receptor (APJ). They are extensively expressed in human and laboratory animal tissues where apelin is involved in a variety of endocrine and paracrine functions. No data concerning their presence and distribution in the salivary glands are present at the moment even if the presence of apelin in human saliva has been shown (1). So, in continuing a study which started a few years ago with the aim of verifying the influence of different physical forms of one diet on the mandibular glands of growing pigs, we tested the presence and distribution of apelin and its receptor in pig mandibular glands and the existence of a variability as a consequence of a more or less intense chewing activity and therefore of the stimulation of the functional activity of the mandibular gland.

Methods: The experiment was conducted using 32 growing pigs fed ad libitum for 4 weeks with one of the four experimental diets. The four diets, identical for chemical and botanical composition, differed in the physical forms: FP - Finely ground pelleted diet (dMEAN, 0.46 mm); CM - Coarsely ground meal diet (dMEAN, 0.88 mm); CP - Coarsely ground pelleted diet (dMEAN, 0.84 mm); CE - Coarsely ground extruded (dMEAN, 0.66 mm) diet. At the end of the experimental period, the animals were euthanized, the mandibular gland specimens were immediately removed and fixed in buffered formaldehyde for 24 h at room temperature and subsequently processed for embedding in paraffin, following routine tissue preparation procedures. The immunohistochemical reaction was visualized on 5 µm sections, collected on poly-L-lysine-coated glass slides, using two primary polyclonal antibodies (anti-AP and anti-APJ), the corresponding secondary biotinylated antibodies, the avidin-biotin-complex and the DAB as the chromogen.

Results: The immunohistochemical study showed a peculiar immunoreaction for AP and APJ in the mandibular glands of the animals examined. In particular, an immunopositive reaction for AP was evident in all epithelial cells of the ducts in the animals fed with coarser diets (CP and CM groups) while in all other animals and in particular in those of the CE group the AP immunopositivity was no longer present. Regarding the APJ receptor, a positive immunoreaction was observed in some of the epithelial cells of the ducts in all the animals examined with the exception of the animals fed with the coarser diet (CM group). Immuno-positivity for AP and APJ was not observed in any other glandular structure or in the sections utilized as negative controls.

Conclusions: These results allow to conclude that AP and APJ are present in the epithelial tissue of the ductal component of pig mandibular gland, with a peculiar cytoplasmatic localization. The number of AP and APJ positive cells seems to be influenced by the physical characteristics of the diets ($P<0,001$) with a positivity that appears to be differently localized in the glands: these observations are in agreement with similar observations made in the previous year for other molecules (2) and lead us to speculate that these variations are strictly related to the functional adaptation of the gland to the more or less intense masticatory activity.

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40.

Comparison of phosphorus digestibility between female and male broiler chickens

Vergleich der Phosphorverdaulichkeit zwischen weiblichen und männlichen Broilern

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Especially for phosphorus (P) availability in chickens, different definitions of available P have been proposed and various approaches are in use. These differences impede the comparison of published data and the compilation of comprehensive feeding tables for the use in practical feeding (1).One opportunity to reduce the variations may be the application of a standardized sampling method based on precaecal P digestibility (1). The WPSA-protocol (1) for the determination of P digestibility specifies to use male broilers of fast growing strains. Nevertheless, the question remains, if the determined digestibility values can also be used for female broiler chicks. Hence, the aim of the present study was to compare the P digestibility between female and male broilers using the proposed WPSA-protocol.

Methods: The study was part of an international P digestibility ring study and was carried out at the poultry research station Wimitz (Äußere Wimitz, Austria). Eight pens per diet with eight male birds per pen each, as well as four pens per diet with 15 female birds per pen each, were used for the study. Three low-P experiential diets were based on soybean meal, maize starch, limestone in variable concentrations in diet A and C. Additionally, soy oil, egg-white powder and sugar (feed grade) were included as feed stuffs with fixed contents. Subsequently, 50% of diet A and 50% of diet C were mixed to produce diet B. P content increased from diet A to C from 2.85 to 4.32 g/kg. The Ca/P ratio was kept constant between diets. Hence the Ca content increased from diet A (4.0 g/kg) to diet C (6.1 g/kg). During the whole study, chickens had free access to the pelleted feed and water. Experimental diets were fed from live day 16 to 25. On live day 26 birds were stunned and sacrificed for digesta collection from the terminal ileum. Sample collection was carried out according to the recommendation of the WPSA (1). Data were subjected to 2-factorial ANOVA using the GLM procedure of SAS, applying a model containing factors diet and sex and their interaction (diet x sex). Orthogonal polynomials were used to determine the effects of increasing P contents in diet on P digestibility. Additionally, a Pearson correlation between treatment means of female and male birds was calculated.

Results: The results show, that an increasing P content in diets decreased the P digestibility linearly for female, as well as for male broilers (Table). No differences were detected between sex and the interaction of diet x sex was not significant. The Pearson correlation showed a high coefficient between female and male chicks (0.987; p = 0.1).

Table:P digestibility (in %)

Sex	Diet A	Diet B	Diet C	SEM	p-value (linear)	p-value (quadratic)	P-value diet	P-value sex	P-value diet x sex
Female	68.2[a]	60.8[ab]	55.5[b]	2.02	0.010	0.742	0.0008	0.979	0.968
Male	68.6[a]	59.6[b]	56.1[b]	1.71	0.002	0.130			

Conclusion: The digestibility of P decreases with increasing P content in diet very similarly between female and male broiler chickens. Therefore, it can be concluded, that P availability values evaluated with male broilers, can also be used for female broilers.

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41.

Effect of the origin (oat, barley, spelt and buckwheat) on praecaecal digestibility of raw starch in pigs with experimentally induced pancreatic exocrine insufficiency

Einfluss der botanischen Herkunft (Hafer, Gerste, Dinkel, Buchweizen) auf die praecaecale Verdaulichkeit roher Stärke bei Schweinen mit experimentell induzierter exokriner Pankreasinsuffizienz

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Exocrine pancreatic insufficiency (EPI) results in maldigestion and malabsorption. Due to lack of compensatory mechanisms the fat digestion is markedly affected (1) while digestion of starch is mostly discussed as being not affected as faecal starch excretion does not occur in case of EPI, even no pancreatic enzyme treatment is performed. Determination of praecaecal (prc) digestibility (dig) indicates that prc dig of starch is impaired as well, depending on botanical source of starch and thermal treatment (2). Reduced prc dig can cause massive gas production due to postileal fermentation (3). As the prc dig of starch differs between healthy controls and EPI patients there is need to test starch of different origin in individuals suffering from EPI. This study aimed to test the prc dig of different sources of raw starch used in human nutrition in the model of the pancreatic duct ligated pig (PL-pig).

Material and Methods: The study was performed in 6 adult female minipigs (Ellegaard). All animals underwent surgery for induction of EPI and fitted with an ileo-caecal fistula. The prc disappearance rate (DR) of raw starch was tested in a screening test model according to (2); all sources of starch were tested in all pigs. The test meal consisted of 150 g of raw cereals to be tested (oat, barley, spelt and buckwheat), 30 g methylcellulose, 25 g of olive oil and 0.625 g chromium oxide. Spelted cereals were used in a dehusked form. As the starch sources were used in equal amounts per meal the different starch content caused slight differences in the amount of ingested starch per meal (see table 1). No pancreatic enzymes were given during the trials. Samples of ileal chyme were freeze dried and starch content was analysed polarimetrically while chromium oxide was analysed according to (4). Student`s t-test was used for statistical analysis (SAS).

Table 1: Starch content and absolute amount of starch per meal of the different test diets used

	Test diet (starch source to be tested + methylcellulose + oil)			
Source of starch	Oats	Barley	Spelt	Buckwheat
Starch content (g/kg dm)	505	513	502	424
Starch per meal (g)	82.1	82.5	80.1	67.9

Results: Prc DR of starch differed markedly; while test diet with oat showed an almost complete prc DR with low variation the prc DR of buckwheat was significantly lower (see table 2). Barley and spelt resulted in an intermediate prc DR not differing from oats and buckwheat (see table 2).

Table 2: Praecaecal disappearance rate of starch in pigs with experimentally induced exocrine pancreatic insufficiency fed diets with different starch sources

	Test diet (starch source to be tested + methylcellulose + oil)			
	Oats	Barley	Spelt	Buckwheat
Prc DR of starch (%)	97.0 ± 1.14 a	88.8 ± 8.20 ab	88.6 ± 8.32 ab	68.9 ± 16.9 b

Different letters mark significant effect of starch origin on prc DR ($p<0.05$); students´s t-test

Conclusion: Although PL-pigs received no pancreatic enzymes, the prc DR of starch was almost complete when raw oat was fed. The low prc DR of buckwheat is worth mentioning as it is supposed to result in an increased gas production when it is fermented in the hindgut. The results are of greatest interest for optimizing dietetic measures for EPI patients in human and veterinary medicine especially as the use of raw cereals is getting more and more popular ("uncooked vegetarian food") in human but also in dog nutrition. To conclude there is a distinct effect of botanical origin on prc DR of raw starch in PL-pigs not substituted with enzymes. Even it is well known that raw oat is highly digested praecaecally in healthy individuals data in EPI patients were lacking up to know.

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42.

Impact of soybean protein on ruminal phenolic content by feeding grass silages containing different levels of true protein *in vitro*

Einfluss von Sojazulagen auf den ruminalen Phenolgehalt bei Grassilagen mit unterschiedlichen Reineiweißgehalten in vitro

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Grass silages containing < 50 % true protein (TP; BARNSTEIN method, VDLUFA III, method 4.4.1.) in total crude protein (CP; KJELDAHL method, VDLUFA III, method 4.1.1.) are suspected to cause severe non-infectious dairy herd diseases in northern Germany (1). In practice soybean meal improved the clinical symptoms. Previous studies *in vitro* have shown an increase of phenolic compounds in bovine ruminal fluid when suspected grass silages (TP/CP < 50 %) were fed. An *in vitro* study was conducted to assess the effect of soybean protein on the ruminal content of total phenolics and o-diphenols during fermentation of grass silages with TP/CP < 50 %. Phenolic compounds are found in all higher plants as secondary plant metabolites with different pharmacological effects depending on the substance. O-diphenols are known to reduce proteolysis in grass after cutting.

Methods: In the experiment 2 x 4 runs with duration of 28 days a time were conducted using the RUmen SImulation TEChnique (RUSITEC). Two different grass silage pairs were tested in 4 RUSITEC runs each (R1-4 and R5-8). During the adaptation and control period (9 days) grass silages with TP/CP > 50 % (CS) and corn starch were fermented in all three fermenter groups (control, I, II). In the following experimental period (10 days) fermenter groups I and II were fed grass silages containing TP/CP < 50 % (TS) and group II with addition of soy protein. During the end period (9 days) the initial diet was added to all fermenters again. The two grass silages compared in each trial were harvested from the same fields. In daily taken samples of ruminal fluid the total phenolic content was measured using the colorimetric FOLIN-CIOCALTEAU method. The content of o-diphenols was quantified with a colorimetric method after performing an alcoholic extraction.

Results: The contents of total phenolics and o-diphenols stabilized in all eight trials during the adaptation and control period. A change of grass silages fed by day 9 resulted in a significant shift (Table 1, paired T-Test) of detected phenolic quantities. The total phenolic content was lowered by addition of TS in R1-4 and increased in R5-8, respectively. O-diphenol levels declined during the experimental period of all trials in fermenter groups I and II but in R5-8 at a lower extend. The effects of TS addition were most visible on days 15 to 19. The soy protein supplementation had no noticeable effect. After switching back to the control ration on day 19, the concentrations of phenolic compounds aligned with the control again within four days. The control values were consistent during the whole trial.

Table 1 Total phenolics and o-diphenol content in groups I and II on days 15 to 19

[% change compared to control]

	Total phenolics		O-diphenol	
	Group I	Group II	Group I	Group II
R1-4	-20,7***	-19,7***	-5,11***	-6,24***
R5-8	+12,8***	+12,1***	-1,89***	-2,31**

* p < 0,05; ** p < 0,01; *** p < 0,001

Conclusion:

It could be shown that grass silages with TP/CP < 50 % had significant effects on the phenolic composition of the ruminal fluid. The mechanism of the beneficial effect of soybean meal seen in practice could not be elucidated in this study. Although there was no explicit monodirectional change in the total phenolic content identifiable, it appears likely that the phenolic composition of a diet can influence the ruminal digestion.

(1) EICKEN, K. (2005): Nutztierpraxis Aktuell 13, 13-16

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43.

Studies on the metabolism of amines when feeding grass silage containing different levels of true protein and the influence of soybean meal using the RUSITEC-System

Untersuchungen zum Stoffwechsel von Aminen bei Einsatz von Grassilagen mit unterschiedlichen Reineiweißgehalten unter Zulage von Sojaprotein im Pansen in-vitro

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Grass silages containing different levels of true protein are a discussed problem in the health management of dairy cows. A ratio of < 50 % true protein in total crude protein content (TP/CP) in grass silages seems to have negative effects on the health of dairy herds when feeding a diet containing more than 50 % grass silage (1). The metabolism of amines in the rumen is closely related to the input of protein and amino acids. Moreover amines have many different effects on the organism, positive as well as negative, depending on the amount and type of amines and the barrier function of the digestive epithelium (2).

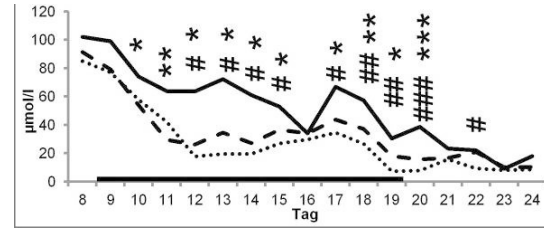
Methods: The RUmen SImulation TEchnique (RUSITEC) is an in vitro system of the rumen, which is often used to evaluate its digestive function on different feedstuff. Eight digestive experiments lasting 28 days each were completed. The experiment consisted of 3 stages. In the initial stage (I) the system stabilized while control silage (> 50% TP/CP) and corn starch were added. Two fermenters stayed with control silage the entire time of the experiment. In the following experimental stage (II) silages of lower TP levels (< 50% TP/CP) and corn starch were added. To evaluate the influence of soy protein (SP) soybean meal was added to two fermenters (Test 2) whereas two stayed without additives (Test 1). The final stage (III) is to evaluate if the system returns to its original balance while the initial control silage and corn starch were fed again. Samples were taken from the RUSITEC-fluid and from the feeding bags after the incubation for 48 h. The content of polyamines was analyzed using the LC-MS technique.

Results: Six amines and their corresponding amino acids were analyzed (Tab. 1). General findings were that the total amount of all analyzed amines in the two fermenters containing the control silage was higher compared to the four test-fermenters containing silages of < 50% TP/CP content (see Putrescine Fig. 1). The results show that the fermentation of silages used in Test 1 and Test 2 containing lower levels of TP in the silage added caused significantly different contents of polyamines in the RUSITEC-system (paired T-test; * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$in Test 1 and # $p < 0,05$; ## $p < 0,01$; ### $p < 0,001$in Test 2, respectively).

Table 1: Amine and corresponding amino acid content during the experiment

Amino acid	**Amine**
Aspartic acid ↓	β-Alanine ↑
Lysine ↔	Cadaverine ↔
Arginine/Ornithine ↔	Putrescine ↔
Arginine/Ornithine ↔	Spermidine ↔
Tyrosine ↓	Tyramine↑
Arginine/Ornithine ↔	Thermospermine ↔

Fig.1: Total amount of putrescine in each stage of the experiment (- Control; Test 1; Test 2)



Conclusion: In the experiment we were able to point out that grass silages with different amounts of true protein have an influence on the metabolism of amines in the RUSITEC-system. Depending on the metabolic cascades, some amines and their corresponding amino acids keep a balance whereas others transform completely into the amine.

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Supported by Milchförderungsfonds Hanover-Braunschweig

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44.

A new scope for rumen simulation technique (RUSITEC): *in vitro* investigation of specific ruminant xenobiotic metabolism by using ovine rumen fluid

Neuer Anwendungsbereich for die „Rumen Simulation Technology" (RUSITEC): in-vitro Untersuchungen zum Fremdstoffmetabolismus mit ovinem Pansensaft

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For registration of plant protection products (PPP) 14C-metabolism studies are required in rat (OECD 417), livestock (OECD 503) and plants (OECD 501, OECD 502). Although some minor differences in xenobiotic metabolism of ruminant species might be present, it is accepted by the authorities (EFSA, EPA, OECD countries) to use one ruminant species to extrapolate to the whole group of ruminants. This is supported by data from industry, where the relevant metabolites obtained in a goat metabolism study (OECD 503) are usually been detected in cow feeding studies (OECD 505).

Occasionally there are specific questions occurring on the ruminant xenobiotic metabolism: 1) are the observed metabolites ruminant specific and formed directly in the rumen? 2) are ruminants able to cleave plant specific metabolites like glycosides to the respective aglycon? In the past new additional in vivo goat metabolism studies with at least one animal were performed to address the open questions.

Literature research identified the rumen simulation technique (RUSITEC) as a potential appropriate method to simulate the rumen and its metabolic behaviour in vitro. The aim of this project was to elucidate if RUSITEC is able to address robustly specific questions on xenobiotic metabolism in ruminants for registration of PPP beyond OECD 503.

Methods: Fresh ovine rumen fluid was incubated in vitro >7 days by using RUSITEC. The conservation of the physiological conditions were proven by measurement of pH and Redox potential. The microflora composition of the rumen fluid and its viability (bacteria, protozoa and fungi) was monitored by microscopy, incubation on agar plates for 24 h at 39°C and the detection of β-Glucosidase (β-Glucosidase Activity Assay Kit MAK129 Sigma-Aldrich Chemie GmbH).

The metabolic behavior and performance of the rumen fluid was tested by e.g. incubating 14C-triazole derivative metabolites (TDM) like triazole alanine (TA); triazole acetic acid (TAA) and triazole lactic acid (TLA), which are usually formed in plants after application of triazole-containing fungicides. It is already known from cow feeding studies (OECD 505), that TA is cleaved into 1,2,4 triazole, while TAA is stable. 14C-TA, 14C-TAA and 14C-TLA were applied to the RUSITEC and their metabolic stability were tested by Radio-HPLC over 96 hours.

The glucosidase activity was tested directly by β-Glucosidase Activity Assay and indirectly by application of 14C-Octyl-β-D-glucopyranosid and 12C-Polydatin by Radio-HPLC and UV-HPLC within 96h, respectively.

Results: The pH (mean pH = 6,70 ± 0,07; n=66) and the redox potential (mean redox potential = -301 mV ± 30; n=66) kept constant within 192 h. The bacteria count kept constant from 120 h (Mean 4,1xE7 ± 0,8, n=4) to 192 h (Mean 4,8 xE7 ± 1,4 per mL rumen fluid, n=4).

Radio-HPLC showed that TA was cleaved within 72 h to 1,2,4-triazole, while TAA and TLA were stable. β-Glucosidase activity was determined at 4,8 ± 1,0 U/L (n=4) between 48-192 h. These data were supported by a fast and complete degradation of 14C-Octyl-β-D-glucopyranosid within 1 h in one unidentified metabolite and a second unknown further degradation product. 12C-Polydatin was transformed completely in the same timeframe into Resveratrol (aglycon of Polydatin).

Conclusion: All vitality tests confirmed that the RUSITEC is a successful tool to maintain sheep rumen fluid for at least 7 days in vitro. The rumen fluid maintained its main metabolic performance by using RUSITEC. BASF identified the RUSITEC method, which is usually used in different areas, as an appropriate method to investigate specific questions on xenobiotic metabolism in ruminants. In future BASF will replace in vivo animal studies on ruminant metabolism studies beyond OECD 503 by performing RUSITEC studies. The method will be included in the common method portfolio of BASF leading to a significant contribution to animal welfare (3R: replacement).

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45.

Effect of unconventional oilseeds provided at two lipid concentrations on rumen fermentation and methane formation *in vitro*

Effekt von unkonventionellen Ölsaaten supplementiert in zwei Fettkonzentrationen auf in vitro-Fermentation und Methanbildung im Pansen

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Methane is naturally formed during digestion in the rumen and in the hindgut of livestock. Dietary measures, such as oil supplementation to ruminant feed, aim to decrease methane formation with little or at best negligibly adverse effects on intake and digestibility and thereby on animal performance. However, when supplemented as pure oils, ruminal fermentation will be impaired. By contrast, oilseeds provide the lipids in a form which is partially protected. The major oilseeds, rapeseed, sunflower seed and linseed have been intensively investigated for their potential to reduce CH_4 emission to some extent *in vitro* and *in vivo*, dependent on plant species, level supplemented and diet type (1). The spectrum is, however, much larger. Here we screened four unconventional seeds for their efficiency in modifying ruminal fermentation and mitigating methane formation.

Methods: Seeds of four unconventional plant species were investigated, namely poppy (*Papaver somniferum)*, hemp (*Cannabis sativa*), safflower (*Carthamus tinctorius*; seeds with and without hull), camelina (*Camelina sativa*). As a well-investigated control oilseed, linseed (*Linum usitatissimum*) was included, both as ground and as extruded seed. Seeds were ground through a 1-mm screen with a centrifugal mill. According to the lipid content of individual seed samples, 3.5% and 7.0% of lipids were supplemented on top of the basal diet consisting of hay and concentrate in a ratio 60:40 (200 mg dry matter (DM)). As another control, coconut oil (*Cocos nucifera*) was applied at the same lipid concentrations. In total four Hohenheim gas test runs were performed, with each treatment incubated in duplicate per run. After incubation for 24 h, total gas production and proportions of CH_4 and CO_2 were measured. *In vitro* organic matter (OM) digestibility was calculated from total gas production and contents of crude protein and total ash of the total diet (seeds and basal diet (2)). The data were subjected to analysis of variance considering seed type, lipid level, interaction and run as effects. Differences among means were considered significant at $p<0.05$.

Results: Compared with the basal diet, there were significant effects of 7% coconut oil on the gas production variables investigated. Accordingly, methane yield in coconut oil vs. unsupplemented control was 32.2 vs. 37.6 ml/g DM and 52.4 vs. 59.7 ml/g digestible OM, respectively. The values found with the oilseeds varied between 30.5 and 35.1 ml/g DM and 53.8 and 59.2 ml/g digestible OM; the latter being not significantly different from either coconut or unsupplemented control. Among the oilseeds, safflower seed with hull, poppy and hemp seed significantly decreased methane yield (ml/g DM) compared to unsupplemented control. Interactions between seed species and concentration were found only for few variables.

Conclusion: Once more the effectiveness of coconut oil in mitigating methane emissions was demonstrated when adding it at 7% of dry matter. The values determined with oilseeds were intermediate between the unsupplemented control and the coconut oil treatment. This is not astonishing because the oil present in ground or extruded oilseeds is partially protected from the rumen microbial activity. Overall, the unconventional oilseeds turned out to be similarly, or occasionally slightly more, effective than linseed in methane mitigation.

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46.

Reticuloruminal pH dynamics in early lactating dairy cows experiencing different lipolysis

Retikuloruminale pH-Dynamik frisch laktierender Kühe mit unterschiedlicher Fettmobilisierung

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Introduction: Dairy cows experience strong deficits in energy and nutrients during early lactation, rendering them experiencing various degrees of fat mobilization (1). Feeding management during this period is crucial to mitigate deficits in energy and nutrients while assuring a smooth metabolic adaptation of the cows. To lessen the energy deficits postpartum, cows usually are fed increasing amounts of energy-rich concentrates. These shifts in the diet composition increase the risk of rumen fermentation disorders (2). Changes of ruminal pH with regard to fat mobilization during the transition period have not been investigated before in cows. The aim of this study was to monitor individual differences in ruminal pH in early-lactating dairy cows experiencing various energy deficits and lipolysis postpartum.

Methods: A total of 30 (12 primiparous and 18 multiparous) pregnant cows were studied from the expected d 14 prepartum until d 21 postpartum. Cows were kept in the same management conditions and were fed the same close-up and fresh lactation diets. Both diets were based on the same ingredients including hay, corn silage, grass silage and ground barley as main energy source and a mixture of soybean-rapeseed meal as protein supplement, as well as mineral-vitamin supplements. The amount of concentrate in the diet was increased daily, reaching inclusion levels of around 40% (DM basis) at d 21 postpartum. Cows were fed in Calan gates and amounts of feed offered and refused were recorded to determine the feed intake by difference. Reticuloruminal pH was continuously measured on the last 3 d of the observation period, using wireless pH-transmitting units (smaXtec animal care sales GmbH, Graz, Austria). Diurnal reticuloruminal pH dynamics and duration during which pH was below thresholds of 5.8 and 6.0 were calculated. Based on serum concentration of non-esterified fatty acids (NEFA) postpartum, cows were retrospectively classified into low (LOW, n = 8), medium (MEDIUM, n = 11), and high (HIGH, n = 11) lipolysis groups, with NEFA levels of < 0.4 mmol/L, between 0.4 and 0.7 mmol/L, and > 0.7 mmol/L, respectively. Statistical analysis was performed using the MIXED procedure of SAS.

Results: Dry matter intake increased after calving, whereby cows of the LOW group tended to show greater overall DMI compared to their MEDIUM counterparts (13.8 kg/d in LOW, 12.6 kg/d in MEDIUM and 13.1 kg/d in HIGH, respectively; P = 0.07). However, there was no difference in the milk yield between the groups (23.9 kg/d in LOW, 25.5 kg/d in MEDIUM and 26.3 kg/d in HIGH, respectively, P = 0.44). Cows with lowest mobilization showed a tendency (P = 0.10) for longer duration of reticuloruminal pH < 6.0 (300 min/d) compared with other groups (105 and 85 min/d for MEDIUM and HIGH groups, respectively). The time duration of pH <5.8 did not significantly differ between the groups (60, 20 and 16 min/d for LOW, MEDIUM and HIGH, respectively, P=0.27). The diurnal pH profile showed that the highest reticuloruminal pH was measured shortly before the morning feeding and the lowest was recorded late after the feeding in the afternoon. Lower pH values were measured in the group LOW (on average 6.2) compared to the other groups (6.3, P <0.01).

Conclusion: The study indicates a drop of reticuloruminal pH in cows with lowest fat mobilization postpartum suggesting an increased fermentation intensity and output in the rumen of these cows, due to their higher DMI.

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47.

Size-dependent particle retention in the gastrointestinal tract of equids? A non-invasive approach

Größenabhängige Partikelretention im Verdauungstrakt von Equiden? Ein nicht-invasiver Versuchsansatz

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Introduction: In ruminants, particulate digesta retention in the fermentation chamber is a size-dependent process. While particle density also has a very relevant influence, it is clear that larger particles are retained considerably longer. Thus, particles are kept in the fermentation chamber until being fermented to a considerable degree. For equids, the role of particle size for retention in the gut is less clear. Potentially there are areas in the gut which could work as a retention mechanism. Dosing of marker particles of defined length is impossible in non-fistulated equids. However, feeding long marker particles and analysing classes of the faecal particle size spectrum separately is an approach allowing conclusions on the presence of size-dependent retention mechanisms. The study quantified potential effects of 1) particle size and 2) marker type on retention time.

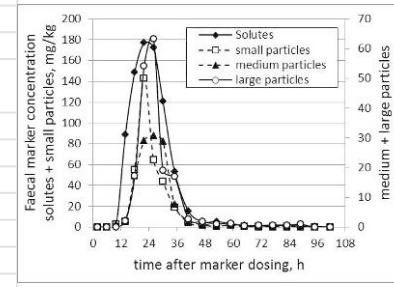
Material and methods: Six Shetland pony mares (body mass (BM) 164 ±31 kg) had ad lib access to hay (Neutral-Detergent fibre: 63% dry matter (DM); CP: 6.3% DM). A mixture of long hay treated with either Cr (mordanted), Ce or La (all particle markers) plus Co-EDTA (marker for solute phase) was fed to the animals in a single dose. Faeces were collected at increasing time intervals (minimum 4 h) for 7 days. They were wet sieved and particle fractions of >2 mm (large), 0.5-2 mm (medium) and 0.063-0.5 mm (small) were analysed separately (inductive-coupled plasma). In addition average faecal particle size was quantified for the ponies by wet sieving and calculating mean particle size. For statistical analysis a mixed model was used (fixed factors: particle size, marker, particle size*marker; random factor: individual) with consecutive comparison of means (Tukey-Kramer).

Results: The ponies ingested 3.25 ±0.60 kg DM of the hay (72 ±11 g/kg $BM^{0.75}$) per day. Mean retention time (MRT) of solutes was 25.4 ±4.1 h. While significant effects of particle size and marker type were found in the statistical analysis (Tab. 1), numerical differences for the MRT of the different treatments were generally small (Fig. 1, Tab. 1). Average faecal particle size was 1.22 ± 0.34 mm.

Tab. 1: Effects of particle size and marker on retention time

Results of ANOVA			
	F-Value		p-value
Particle size	9.62		0.0004
Marker	29.05		<0.0001
particle size x marker	0.67		0.6169
Comparison of means (LSmeans)			
Particle size	large	medium	small
MRT, h	25.1[a]	24.7[ab]	24.3[b]
Marker	Cr	Ce	La
MRT, h	25.5[a]	24.3[b]	24.4[b]

Fig. 1: Faecal marker concentration (in DM)



Conclusions: Marker quantification in separate particle size classes allows investigations on the presence of a particle size dependent retention mechanism in non-ruminating herbivores like equids. While some differences were found in this study, they were on a level of limited biological relevance (e.g. < 1 h difference between large and small particles). As already shown in other studies, retention time of solutes was on a comparable level as that of particles. Average faecal particle size largely confirms data of 1.24 ±0.22 mm for equids (4 species/breeds) on an ad lib. hay diet (1).

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48.

Preparation of boluses for administration of dietary markers to horses and investigations on their suitability

Herstellung von Boli zur Verabreichung von Futtermarkern an Pferde und Untersuchungen zu deren Eignung

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In equine nutrition studies, external markers possibly combined with internal ones might be useful or are required to estimate forage intake, digestibility, faecal output and passage rate. Nevertheless, oral administration is a challenge because of the high sensibility and selection skills of the animal species in question. We hypothesized that it is possible to develop a bolus matrix adapted to the specific conditions ensuring constantly high acceptance and sufficient resistance to microbial spoilage.

Methods: Boluses were made of oat flakes, sugar beet syrup and wheat flour (1:0.8:0.6 by weight). They had been labelled (L) with synthetic wax, made of *n*-alkanes ($C_{28}H_{58}$, $C_{32}H_{66}$ and $C_{36}H_{74}$) as example marker, embedded into filter paper (FP) or hypromellose capsules (HC), and tested against corresponding placebos (P). Boluses were baked (100 °C, 30 min) or freeze-dried. The freeze-dried boluses varied in size (1.5, 2 and 3 cm Ø) and drying time (DT, 6, 12, 24 and 48 h). In one of two bending tests (BT1 and BT2), each P type was loaded up to breakage in a three-point bending configuration (short-beam test) determining the specifically required force for breaking (FL, flexural load) to estimate the horses' required masticatory forces (MF). Acceptance was tested in two randomized cross-over trials (AT1 and AT2) using altogether 34 horses. Acceptance scores were 1: ingested, 2: crumb losses, 3: marker losses (AT1) or denied (AT2), 4: denied (AT1). Freeze-dried P were stored 1 month in a climatic chamber (ST, storage test, 16 h/d: 20 °C, 8 h/d: 16 °C, relative humidity: 65 %), each in a closed box to prevent absorption of humidity, before subsamples had been analysed for residual moisture (RM) and spoilage-indicating microbes referring to VDLUFA methods 28.1.1 to 28.1.4 and 3.1, respectively (1). For statistical analysis (SAS 9.4), unpaired t-test (BT1) and MIXED (BT2, AT and ST) were used with $P < 0.05$ as level of significance. For the latter, least squares means were estimated for bolus types and compared among one another.

Results: The HC might be preferable to FP due to surmised rapid marker release in contact with gastric fluid. Alkane residuals found in the outer matrix shell suggested that baking led these markers melt and partly traverse out of inner FP or HC. This was not evident in freeze-dried variants. Acceptance of L (scores $\leq 1.7 \pm 0.18$, AT1), baked P (scores $\leq 2.2 \pm 0.35$, AT1) and dried P (scores $\leq 1.1 \pm 0.31$, AT2) was constantly high. This was explained by BT, obtaining mean FL of 202 ± 16.5 N for baked P (BT1) and up to 257 ± 22.5 N (3 cm Ø, 24 h DT) for dried P (BT2), which comes close to MF in horses associated with the closing stroke (2). Mastication of L is expected to be easier than of P, because of lower shell thickness. The RM declined with increasing DT but decreasing bolus diameter. It seems to be justifiable to limit DT to 24 h, especially for boluses with no more than 2.0 cm Ø. Even after 6 and 12 h DT, RM was 7.5 ± 0.52 (1.5 cm Ø) and 5.7 ± 0.52% (2 cm Ø), respectively, which make the risk of microbial spoilage appear low (3). Indeed, investigated boluses were unspoiled at least up to 1 month after preparation.

Conclusion: During the study, a bolus meeting the particular requirements has been developed. Variable bolus sizes enable it to be applied to various marker dosages and thus also to be used in different target animals. The freeze-dried matrix type is also open to be used with other thermo-labile markers, or such with thermo-labile coatings, and therefore to a broad range of markers or other substances.

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49.

Use of synthetic alkane waxes as single- or multi-component dietary markers in farm animals

Nutzung synthetischer Alkanwachse als Einzel- oder Mehrkomponentenfuttermarker bei Nutztieren

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Synthetic alkanes can be applied as external markers to estimate faecal output, digestibility and passage kinetics. Furthermore, they are easy to combine with plant alkanes for more sophisticated investigations e.g. on intake of feedstuffs of distinct biological origin. Successful application requires accurate and uniform labelling of boluses or feedstuffs and this in turn secure handling of markers during preparation. We hypothesized that melting synthetic alkanes to wax might enhance accuracy and uniformity of subsequent bolus labelling and further simplify it. This is particularly required when multiple alkanes are combined or large quantities of boluses are needed. The aim of the study was to test a procedure of preparing alkane waxes on model scale by use of a portion of ~ 1:300 basing on 150 mg per bolus, which is a dosage recommended to be applied in large livestock for an administration two times a day (1).

Methods: Using $C_{28}H_{58}$ (C28), $C_{32}H_{66}$ (C32) and $C_{36}H_{74}$ (C36) synthetic alkanes, three single-component waxes, three binary waxes (C28:C32, C28:C36, C32:C36) and a tertiary mixed wax were produced with five repetitions each. To assess the impact of melting and crystallization, the individual alkanes were analysed by gas chromatography (GC) in untreated crystals, crystalline mixtures and the finished model waxes. Additionally, the thermic properties of $C_{24}H_{50}$ (C24) to $C_{38}H_{78}$ (C38) even-chain alkanes were studied by thermogravimetry with consistently increasing temperature and under isothermal conditions (180 °C, 20 min), respectively. Statistical analysis was performed with SAS 9.4 REG using linear regression analysis to study the relationship between alkane contents in crystal- or wax-samples that were originally weighed with those measured by GC and to determine the specific difference of the slope of respective regression lines by one. The level of significance was pre-set at $P < 0.05$. The relative difference (RD) by one is given as mean ± standard deviation among the repetitions for each sample variant.

Results: Depending on chain length (CL) and thus molecular weight of alkanes, weight reduction by emergence of soot during melting started between 176 °C (C24) and 227 °C (C38) and further increased rapidly. Throughout isothermal treatment, weight loss from alkanes was lowest with highest CL (0.3 %) and *vice versa* (23.7 %). Differences between weighed and *via* GC measured contents of crystalline alkanes were not significant, except for single C36, where the measured quantities were always higher than the weighed ones ($P = 0.019$). Weighed and measured contents of individual alkanes in single-component and total alkanes in multi-component waxes were similar with a RD of at most 6.6 ± 5.5 %. The RD between weighed and measured contents of individual alkanes in multi-component waxes was maximally by 47.4 ± 25.7 % and was highly variable. Unexpectedly, RD was low for C28 (5.9 ± 5.8 %) and C32 (5.7 ± 4.3 %) in their combined binary waxes.

Conclusion: Synthetic alkanes are thermo-labile why exposure to high temperature during preparation of boluses or labelling of feedstuffs need to be assessed critically. Reasons for that might be i) complex disorders of the conformation of alkane molecules particularly during melting of alkane mixtures, and ii) apparently incomplete separation following re-crystallization. This may lead to displacements within waxes, which cannot be foreseen or quantified, and thus loss of their suitability as dietary markers. Alkane recovery from binary waxes of C28 and C32 was unbiased on model scale and whether this can be confirmed on original scale (1) needs to be validated further. Nevertheless, for practical use the preparation of waxes might be beneficial because the handling is easier than that of alkane crystals.

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50.

The effect of methane on properties of digestive physiology in dairy cow

Effekt von Methan auf Merkmale der Verdauungsphysiologie bei Milchkühe

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Due to the contribution of enteric methane (CH_4) as a greenhouse gas, its production is considered an undesired (but partially unavoidable) side-effect of herbivore digestion. Digestive processes associated with the quantity of feed eaten can affect changes in the rumen and whole tract residence times, and consequently contribute to differences in CH_4 emissions. Previous work has however suggested that CH_4 itself may influence the physiology of the gastrointestinal tract (GIT), in particular by slowing down digesta passage (1,2). Therefore, we investigated the effect of adding CH_4 to, or removing it from, the reticulorumen (RR) of non-lactating dairy cows.

Methods: In a 4x4 Latin square experiment with 4 rumen-fistulated dry Holstein cows (~700 kg body mass), 4 treatments were tested: (i) control (C); insufflation (295 ± 82 L/d) of either (ii) CH_4 gas (iCH_4) or (iii) N_2 gas (iN_2) via fistula; and (iv) reduction of CH_4 production via the application of bromochloromethane (BMC) twice daily. Dry matter intake (DMI), apparent total tract digestibility, digesta mean retention times (MRT) of four different- markers, rumen motility, rumination activity, CH_4 dissolved in rumen fluid (CH_4RRf), as well spot samples of breath CH_4 emission (determined thrice daily using GreenFeed) were measured for 1-week after 1-week of adaptation to treatments. Each period had 2 weeks recovery between treatments. Cows were fed chopped grass hay (neutral detergent fibre [NDF], 592 g/kg dry matter) for ad libitum intake and limited amounts of concentrate and mineral feeds. Data were analysed by mixed models including treatment (or, alternatively, CH_4RRf), DMI relative to body mass$^{0.85}$ (rDMI), period and individual cow. Most measurements were influenced by rDMI, so this was included as a cofactor.

Results: Dry matter intake, which averaged 29 ±5 g $kg^{-0.85}$ d^{-1}, was significantly affected by treatment (P=0.024), and was lowest on BMC. Breath CH_4 emission was highest for iCH_4 and lowest for BMC (P<0.001), but the only treatment that affected (reduced) CH_4RRf was BMC (P<0.001). Controlling for rDMI, CH_4RRf had a significant negative effect on MRT in the GIT (P=0.001-0.052, depending on the marker) (but not in the RR, P>0.10), i.e. digesta passage was faster in the presence of CH_4. CH_4RRf also had a significant negative effect on NDF digestibility (P<0.001), and on time spent ruminating (P=0.013) and chews per bolus (P=0.023). Treatment significantly affected the number of rumen con-tractions per minute (P=0.010; lowest on BMC) and the interval between contractions (P=0.038; highest on BMC).

Conclusion: The results have to be interpreted with caution because CH_4 insufflation did not achieve a change in CH_4RRf; changes in CH_4RRf were achieved by the BMC treatment only. In contrast to expectations, higher levels of CH_4 were associated with a shorter MRT in the whole GIT and not a delay in digesta passage. This also affected RR motility; however, the main effect on MRT apparently occurred beyond the RR. It has to be corroborated in future studies whether there actually is a feedback mechanism by which high levels of CH_4RRf (and hence also in fluid passing from the RR into the lower digestive tract) induce a higher GIT motility to avoid prolonged digesta retention. Most likely, such a putative effect is different from general differences in MRT found between high- and low-CH_4 producing animals (3).

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51.

Characterisation of the structural and functional diversity of the rumen microbiota in dairy cows

Charakterisierung der strukturellen und funktionellen Diversität der Pansenmikrobiota von Milchkühen

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The structure and functions of microbiota inhabiting the rumen are mainly shaped by the host's diet. Apart from the dietary impact, individuals maintain their own specific gut microbial composition. Describing the influence of different diets on the inherent community arrangement and associated metabolic activities of the most active ruminal groups (composed of bacteria and archaea) is of great interest for animal nutrition, biotechnology and climatology.

Methods: Samples were obtained from three ruminally fistulated lactating Jersey cows rotationally fed with three rations. The rations contained 52% concentrate (36% wheat, 36% barley, 14% soybean meal, 12% molasses, 2% mineral mix) and 48% roughage. The roughage was maize silage (MS), grass silage (GS) or meadow hay. Samples were taken from three sections (ruminal fluid, squeezed solid and solid matter). DNA was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedical). Illumina sequencing of the 16S rDNA (V1-V2 regions) [1] was used to characterize the overall bacterial diversity. In addition, samples were prepared for protein extraction using a cheesecloth-based protocol [2]. Proteins were extracted and further purified by a short 1D gel electrophoresis step. Peptides were created by an in-gel based trypsin digestion and measured by liquid chromatography coupled to mass spectrometry (LC-ESI-MS/MS, Thermo Scientific Q Exactive Plus system). Peptide and protein identification was done using Thermo Proteome Discoverer and NCBInr public gene database for proteins of bacteria, archaea and protozoa. In addition, the respective metabolomes of the rumen fluid samples were determined by 500MHz-NMR spectroscopy.

Results: Phylogenetic data analysed by sequencing the 16S rDNA showed a clear separation between the sampled sections and predominating individuality of the microbiota structure of each animal. Significant alterations in response to the roughage source were observed when metaproteomics and metabolomics data were explored. Species of the family *Succinivibrionaceae* showed higher abundance after feeding MS whereas cellulolytic *Fibrobacteraceae* appeared in larger numbers in GS and hay-based diets. Fibre-degraders of the *Lachnospiraceae* family were found in great quantities in the solid fractions. Members of the *Erysipelotrichaceae* family known to be higher in fat-rich diets were increased exclusively upon feeding hay as the roughage. Comparing 16S rDNA based results with metaproteomic analyses, *Prevotellaceae* were found to be more abundant in the protein data. In contrast, proteins belonging to *Acidaminococcaceae* and *Ruminococcaceae* appeared to be less contributing. Enzymes involved in amino acid transport and metabolism increased after feeding the MS diet, proteins related to posttranslational modifications, protein turn-over and chaperons were less present after feeding hay.

Conclusion: Disregarding the diet-introduced changes in structure and function of the microbiota a host dependent microbiota composition was found to be prevailing. The microbial community of solid rumen matter was shown to be clearly different from rumen fluids. The combination of Omics-technologies represents a powerful tool to investigate the microbiota of complex ecosystems like the rumen. In order to retrieve deeper insight into the complicated network of gut microbial adaptation and to improve utilisation efficiency in livestock feeding further investigations will be necessary.

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52.

Evidence for restored ruminal fibre degradation in response to a long-term subacute ruminal acidosis challenge

Indikation für einen wiederhergestellten Faserabbau im Pansen als Reaktion auf eine langfristige subakute ruminale Acidose

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During subacute ruminal acidosis (SARA) ruminal pH falls to less than 5.8 for several hours per day. Fibre-degrading microbes are sensitive to low pH and SARA is viewed to be detrimental to ruminal fibre degradation. However, cows with a low ruminal pH can maintain normal populations of cellulolytic bacteria, depending on SARA conditions (1), suggesting the importance of duration and pattern of high grain feeding for ruminal microbial adaptation. This study investigated the possibility for ruminal fibre degradation to be restored during the course of a long-term SARA challenge.

Methods: Eight rumen-cannulated non-lactating Holstein cows were blocked by body weight and randomly assigned to 2 SARA models, namely transient and persistent SARA. The experiment consisted of 2 runs (each n=8). In each run, all cows started at baseline and were fed a forage-only diet, followed by a 6-d transition to a 60% concentrate diet to induce SARA. Cows with persistent SARA were then challenged continuously for 4 wk. Transient SARA animals had 1 wk of SARA challenge, then a 1-wk break (fed only forage) and then 2 wk of re-challenge. Ruminal fibre degradations of grass silage at 0, 4, 8, 24 and 48 h of incubation were measured by in situ technique. For transient SARA, the degradation was determined at baseline (wk0) and 4 days after the concentrate break (coded as SARA wk3). For persistent SARA this was done at baseline (wk0), after 1 wk of SARA challenge (SARA wk1) and at the end of SARA (SARA wk4). In situ samples were analyzed for DM (2 runs), NDF and ADF (1 run) contents and the degradation was calculated. Samples were also analyzed for abundances of total bacteria, fungi and cellulolytic bacteria using quantitative PCR. Measurements at all periods were treated as independent treatments and were analyzed using Proc Mixed of SAS. The statistical model tested treatment effect and took into account repeated measures of incubation times and, in case degradation data, covariate of 0-min incubation.

Results: At 48 h of incubation, about 70% of DM and 50% of the fibre fractions were degraded. Pairwise comparisons among treatment means indicated that 24- and 48-h NDF and ADF degradations of SARA wk4 were similar to those of baselines, while those of SARA wk1 and wk3 decreased the fibre degradation (20-29%) compared with baseline values (P<0.05). Degradation of DM showed a similar trend; but SARA wk3 did not differ from the baseline. Bacterial abundances increased with progressing incubation by 2 log units (0h vs 48h). Treatment did not affect total bacterial gene copies but SARA treatments clearly decreased total fungi compared with baseline values (P<0.05). Changes in relative abundances of *Fibrobacter succinogenes, Ruminococcus flavefaciens* and *Ruminococcus albus* did not clearly explain the effect of SARA treatments on decreased fibre degradation. Relative proportion of *Butyrivibrio fibrisolvens* negatively responded to SARA treatments which were, on average, 0.2 fold of the baseline of persistent model (P<0.05).

Conclusions: Lowering of the degradation of fibre fractions was already seen after 1 wk of the high concentrate feeding used to induce SARA. However, extending the feeding duration can restore the fibre degradation. Other fibrolytic bacteria than the species studied might have contributed to fibre degradation in the rumen or changes in fibrolytic activity were mostly on functional level.

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53.

Spatial variation in the gut microbiota of broiler chickens fed diets supplemented or not with calcium, phosphorus and phytase

Variation der Zusammensetzung der Mikrobiota im Verdauungstrakt von Broilern bei Einsatz eines Mischfutters ohne oder mit Ergänzungen von Calcium, Phosphor und Phytase

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The chicken gastrointestinal microbiota is highly impacted by the diet. Presence or absence of specific bacterial species may influence a healthy gut microbial balance and enzymatic hydrolysis of certain feed fractions. Phytase supplementation improves phosphorus (P) digestibility and reduces P excretion of broilers. However, cleavage of P from phytate due to added phytase is affected by the level of P and calcium (Ca) in the diet. Supplementation of diets with Ca and P can affect the composition of the microbial community in the digestive tract of broilers [1]. In this study we aimed to distinguish the microbial communities of digesta and mucosal samples from three sections of the GIT of broiler chickens fed diets differing in mineral P, Ca and microbial phytase supplementation.

Methods: A maize-soybean meal basal diet (3.7 g P/kg and 5.6 g Ca/kg) was supplemented or not with P (2 g/kg), Ca (3 g/kg) and phytase (1500 FTU/kg of feed of an *E. coli*-derived phytase) and used in pelleted form in a 2 × 2 × 2 factorial arrangement of treatments. Broilers were allocated to 56 floor pens in groups of 19. Birds were fed with a commercial starter diet without coccidiostats until day 14 and then the pens were randomly assigned to the 8 dietary treatments. Digesta and mucosal samples from crop, ileum and caeca were taken at day 26 from individual birds after being euthanized by carbon dioxide asphyxiation and the digestive tract dissected. Total nucleic acids were extracted using a commercial kit from individual bird samples and then subjected to 16S Illumina amplicon sequencing. Phylogenetic analysis of the 16S rRNA gene sequences was assessed using RDP pipeline [2] and statistical analysis using Primer 6 [3].

Results: A high variability in the microbial composition was observed between individuals (4-6 birds) within sections and diets. The average similarity ranged from 29-81% in the crop, 18-48% in the ileum and 16-38% in the caeca for both digesta and mucosa samples. An effect of each diet on each section was observed on mucosa samples (p=0.003). Such effect was not found in the digesta samples, however a significant difference between the diets was detected in the crop and caeca sections. *Ralstonia* was also observed across all diets on average abundance of 0.05-15%. Ileum digesta samples were mainly colonized by *Lactobacillus*. *Clostridium* XI and *Streptococus* were also observed in ileum digesta with higher abundance (>50%) in diets with addition of P and no addition of Ca. In mucosa samples four main genera were detected: *Lactobacillus*, present in all the samples, *Clostridium* XI, *Ralstonia*, and *Parvimonas* (more abundant in diets with P and Ca supplementation). The highest diversity was found in caeca especially in mucosa samples. The most abundant families detected in caeca were *Anaeroplasmataceae* (less abundant in diets with Ca and P or phytase addition), *Erysipelotrichaceae*, *Lachnospiraceae*, *Peptococcaceae* 1 and *Ruminococcaceae* contributing for more than 71% of total abundance of digesta and mucosa samples.

Conclusions: Diets had relatively minor effects on the microbial community, this might be because of the high variability observed among birds analysed for each diet and section. In the ileum the diets supplemented with P and Ca increased the abundance of *Parvimonas*. Diet with phytase and no Ca and P addition decreased the abundance of *Anaeroplasmataceae* in the caeca.

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54.

Digestibility of calcium and phosphorus in fattening pigs (finishers) fed exclusively with whole-plant corn silage vs. compound feed

Ca- und P-Verdaulichkeit bei Schweinen [Endmast] nach ausschließlichem Einsatz von Mais-Ganzpflanzensilage im Vergleich zu einem üblichen Alleinfutter

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In former studies (1) the apparent digestibility (aD) of nutrients of whole-plant corn silage (WPCS) in fattening pigs was determined. WPCS is an affordable fiber source for pigs that can be used to elevate the crude fiber supply. A new technique of wet-grinding after soaking allows the use of WPCS in pork producing units equipped with liquid feeding systems without technical problems i.e. clogging of the manure system due to long fibrous particles. Higher dietary levels of fiber might contribute to animal welfare, reduce abnormal and aggressive behavior in pigs and improve the gut health (2). In this approach the WPCS was fed exclusively ad libitum to test the aD of calcium and phosphorus.

Methods: In experimental period one 3 barrows (BW 68.3 ± 5.65 kg, age 169 ± 6.70 days) were housed in individual pens and fed restrictively (ca.1.4 of maintenance requirement of energy) with a common compound feed [881 g DM/kg; per kg DM: 40.9 g CF, 6.85 g Ca, 5.01 g P; Ca and P-sources: Calciumcarbonate - $CaCO_3$, Mono-Calciumphosphate - $Ca(H_2PO_4)_2$ and Mono-Dicalcium-Sodiumphosphate; The mineral feed, which was part of the concentrate (3.1% of mass), contained 45.000 IE of Vitamin D_3 and Phytase]. After a 5- (WPCS:10 days) day period of adaptation to the diet, the total collection of faeces was performed the following 5 days. In experimental period two a wet-ground WPCS was fed exclusively ad libitum to the same but heavier / elder animals (BW 177 ± 4.85 kg, age 350 ± 6.70 days). The soaked and wet-ground WPCS contained 76.2 g DM/kg FM and per kg DM: 137 g CF, 4.68 g Ca, 4.06 g P. The content of calcium and phosphorus was determined by atomic absorption/colorimetrically. Statistical analysis was performed with ANOVA-procedure in SAS 9.3 for Windows. The results of both experimental periods were compared and the inevitable endogenous faecal losses of Ca and P were determined via linear regression.

Results: The aD of Ca and P was lower in WPCS but only the rates for Ca differed significantly. The intake of minerals plotted against faecal output (extrapolation - zero intake) resulted in daily endogenous faecal losses (EFL) of 9.59 for calcium and 7.29 mg/kg BW for phosphorus. Values in the literature: [(3) 20 mg/kg BW/d total endogenous loss of calcium; 7-10 mg/kg BW/d total endogenous loss of phosphorous]. The values of the calculated true digestibility differed significantly. The calculated true digestibility of Ca and P in the concentrate and the WPCS seemed to be equal. Only if calculated with literature values (3) there was a significant difference in the Ca digestibility. The intake of each element was plotted against its faecal loss. In this case the slope of the regression line multiplied by 100 represents the percentage of undigested element (4): Ca (54.0%) P (45.1%). Ca: $y = 0.5401x + 9.5954$; P: $y = 0.4511x + 7.2902$. The digestibility which results out of this values (Ca: 45.9/P: 54.9) is consistent to the values determined (Ca: 45.8-46.0 and P 54.7-55.0%).

Conclusion: This study showed that the true digestibility of Ca and P in WPCS is comparable to values of common compound feed, based on cereals, soybeanmeal and a mineral feed, even if WPCS was fed exclusively without supplementation of a mineral feed. The concentrate contained sufficient Ca and P, therefore an underestimation of fecal Ca and P losses due to counter regulation in the Ca/P homeostasis could not be ruled out furthermore there was a significant time-delay between the two experimental periods, which should be considered cautiously. The determined values of the aD were in all cases positive, hence the inevitable EFL had to be lower than the total amount of each element. It could be assumend that the roughage WPCS could cause abrasive effects on the mucosa, which would raise the EFL of Ca and P. But this was not observed, because the values for the EFL tended to be lower than in the literature. A true digestibility of Ca ~45% and P of ~55% favor the use of WPCS in pig nutrition.

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55.

Sulphur and Sulphate contents in the digesta of chicken related to dietary intake

Schwefel- und Sulfat-Gehalte im Inhalt des Verdauungstraktes von Broilern in Abhängigkeit von den Gehalten im Futter

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Introduction: Regarding the dietary intake of sulphur containing compounds in poultry there is a marked variation depending on ingredients, mineral compounds, S containing amino acids and of further feed additives. Especially due to rapeseed byproducts and sulphate containing feed additives the dietary intake may vary in a large extent. The fate of ingested sulphur is determined by absorption, degradation, transformation and by excretion (predominantly as sulphate in the urine). Sulphate is known for its acidifying and laxative properties (at least in some species)(1). Here the primary question was whether the dietary sulphur and sulphate intake might interfere with the composition of digesta and excreta (dry matter content?) and thereby result in effects on foot pad health.

Methods: 120 broilers (Ross 708) of mixed sex were randomly distributed on groups of 20 animals in 2m2 sheds from the seventh day of life. Group 1 and 2 were fed with diet A, a cereals based soybean meal compound feed (SBM). Group 3 and 4 got diet B: SBM but including 14.5 % rapeseed meal. Group 5 was fed with diet C, SBM including 14.5 % rapeseed meaal and additionally 1.08 % CaSO4. The broilers were fattened up to 45 days of age. Fresh digesta of the proximal and distal small intestine, caeca and colorectum were taken from 3 broilers of each group in the dissection. The dry matter content (DM), sulphur and sulphate content were analysed in digesta of the proximal and distal small intestine, caeca, colorectum and in excreta (pooled samples of a group). Correlations between sulphate and DM contents in digesta and excreta were analysed by the SAS software (Pearson and Spearman $p \leq 0.05$).

Results: The S contents in the digesta varied markedly – depending on the dietary intake but to a higher extent on the localization within the gastrointestinal tract (GIT). Up to now the high S concentrations in the cranial part of the small intestine cannot be explained. The highest contents of S and SO4 were found in caecal digesta. As observed in other species the predominant compound of S excretion is the sulphate. There was no significant correlation between sulphate and DM contents in the digesta of the distal small intestine, caeca and colorectum. The same was true for the excreta. But, there was a significant negative correlation between sulphate and DM contents in the digesta of the proximal small intestine for broilers fed diet C in contrast to those fed diet A and B.

Table: Sulphur and Sulphate contents in the digesta (day 45) and excreta (day 39) of broilers (g/kg DM)

Diets		prox. small intestine #	dist. small intestine#	caecum ##	colorectum##	excreta###
SULPHUR						
diet A	2.69	8.33 ± 0.718 (7.24-9.14)	4.21 ± 0.500 (3.41-4.68)	8.15/8.74 (3|3)	3.18 (5)	2.90/3.01
diet B	3.10	9.28 ± 1.02 (7.90-10.7)	4.44 ± 0.457 (4.07-5.00)	11.0/11.1 (2|2)	3.59/4.45 (1|2)	3.44/3.98
diet C	4.92	9.93 ± 1.93 (8.52-12.1)	5.26 ± 0.228 (5.00-5.40)	11.2 (3)	6.10 (2)	8.21
SULPHATE						
diet A	2.64	8.35 ± 0.655 (7.44-9.21)	7.57 ± 0.827 (6.31-8.53)	14.7/14.7 (3|3)	6.27 (5)	6.26/5.99
diet B	2.83	11.2 ± 2.07 (8.28-14.6)	8.62 ± 1.35 (6.30-10.1)	17.9/18.0 (2|2)	7.44/8.09 (1|2)	8.52/9.07
diet C	9.07	12.7 ± 3.07 (9.77-12.5)	9.81 ± 1.12 (8.67-10.9)	18.3 (3)	13.3 (2)	21.2

= samples from individuals (line 1: mean and standard deviation; line 2: min-max)

= pooled samples; in brackets: number of pooled individuals; ### = one pooled sample per group (i. e. from all individuals)

Conclusion: The marked differences in the S and SO4 contents of the digesta depending on the localization are related to processes of absorption (prox. vs. dist. parts of small intestine), and secretion (for example via urine; colorectum vs. excreta). Further investigations on digestibility are necessary to understand the fate of S containing compounds in the GIT of chicken.

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56.

Influences of differently processed feather meal in diets for dogs on nutrient digestibility and faecal quality

Einfluss von unterschiedlich verarbeiteten Federmehlen im Mischfutter für Hunde auf die Verdaulichkeit der Nährstoffe sowie die Kotbeschaffenheit

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Introduction: Increasing consumption of poultry meat in human food entails increasing amounts of poultry slaughter by-products that can be an option to fill the demand for animal protein sources in the pet food industry. Feather meal is characterized by high protein contents but its dietary value depends on the extent of hydrolysis. During the last years especially more gently drying techniques were installed to increase the dietary use. Substituting a commercial diet for dogs with 33 % of feather meal reduced the apparent digestibility of crude protein significantly and resulted in unfavourable faecal consistency although being hydrolyzed before [1]. Because of some differences between products of different processing methods the question was whether a reduced amount of supplemented feather meal would minimize the negative effects on digestibility.

Methods: In a cross over model six adult female beagles (eight - ten years of age) were fed a basic diet (extruded compound feed; CA: 62.1g; CP: 280g; CL: 200g; NfE: 429g per kg DM). The dogs were housed individually in kennels and received 300g diet per day. After measuring the apparent digestibility (aD) of the basic diet it was replaced by one of two feather meal products differing in processing (FeMf (finely ground; processing: 80 sec at 270°C + 120 sec at 80°C)/FeMc (coarsely ground; processing: 60 min at 160°C drying); CA: 17.6/13.4g; CP: 931g; CL: 65.1/67.0g per kg DM) by 20%, the finely ground product further by 10 and 5%. Faeces were collected completely after an adaptation period of 5 days and the consistency was scored (1=very hard; 2=solid, well formed; 3=soft, still formed; 4=poor consistence; 5=watery diarrhoea). Furthermore, DM-content, pH-value, and total mass of faeces were monitored. The aD of the feather meal products was calculated in a differential trial. Statistical analyses were done using the SAS 9.3 software (analysis mixed models, respectively, $p \leq 0.05$).

Results: Substituting a diet with FeM (20%) affected the aD as well as the faecal consistency negatively. The aD of the diet was slightly reduced when 20% FeMf were used but the reduction was less than with feeding the coarsely ground version. In the dose effect studies a minimized effect was seen with reduced proportion of the FeMf.

Trial	**I**	**II**	**III**	**IV**	**V**
Basic diet (%)	100	80	80	90	95
FeM_c (%)	-	20	-	-	-
FeM_f (%)	-	-	20	10	5
nutrient content (whole diet; g/kg DM)					
Crude protein	280	410	410	345	313
Crude ash	62.1	52.4	53.2	57.7	59.9
Ca	8.08	7.01	7.37	7.72	7.90
P	6.86	5.91	6.01	6.44	6.65
aD (whole diet; %)					
organic matter	87.2^{aA} ±1.05	82.2^{b} ±1.73	85.5^{cB} ±2.62	85.7^{B} ±1.60	85.6^{B} ±2.19
crude protein	82.4^{aA} ±1.67	74.6^{b} ±3.80	81.1^{aA} ±3.74	80.5^{A} ±3.21	80.8^{A} ±3.23
crude fat	96.5^{aA} ±0.84	93.9^{b} ±0.95	95.0^{cB} ±1.34	96.2^{A} ±0.62	96.0^{A} ±1.20
Faecal parameter					
total mass (g DM/d)	45.5^{aA} ±3.13	57.8^{b} ±4.90	48.0^{cB} ±7.84	48.6^{B} ±4.68	50.5^{AB} ±8.06
DM (%)	31.1^{aA} ±2.56	29.9^{a} ±3.09	27.9^{bB} ±2.46	29.0^{B} ±2.10	30.8^{A} ±1.83
Score	2.11^{aA} ±0.11	3.63^{b} ±0.24	3.86^{bB} ±0.86	3.43^{C} ±0.43	2.60^{D} ±0.26

$^{a, b, c}$indicate significant differences ($p \leq 0,05$) between trial I, II and III; $^{A, B, C, D}$indicate significant effects ($p \leq 0.05$) of FeMf at different dosages

Conclusion: FeMf or FeMc show an aDos of 79.2 or 64.0% and a content of 742 or 617 g digestible CP as well as 16.4 or 13.7 MJ ME per kg DM (differential trial). In comparison to older studies todays processing enhanced digestibility of the faether meal markedly. Although both products were hydrolyzed, drying temperature and drying time seem to have a further impact on the aD with marked advantages to the product shortly dried with high temperature. Furthermore, the results show that application quantity has a great effect on the aD as well as on faecal parameters.

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57.

Trace element balances with different Cu-, Zn- and Se-supply in horses at maintenance

Spurenelementbilanzen bei Pferden im Erhaltungsstoffwechsel in Abhängigkeit von der Cu-, Zn- und Se-Versorgung

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In 2014 the Society of Nutrition Physiology published new recommendations regarding the energy and nutrient supply of horses (1). Nowadays the daily recommended amount of trace elements is related to the metabolic body size instead of dry matter intake. The aim of this study was to verify the recommended - theoretically derived - dietary trace element levels in feeding studies at maintenance level with a high proportion of roughage in the ration and at identical Ca-, P- and Mg-intake.

Methods: Three pony geldings (BW: 379 kg ± 29.2; age: 4, 6 and 8 years) were housed individually and fed a diet meeting the maintenance requirements according to the GfE 2014. After 10 days of adaptation, the ponies were put in stalls (1.05 m*1.65 m) to enable a total collection of faeces and urine over a period of 10 days (5d of collection, 2d of regeneration, 5d of collection). At the beginning (d11) and at the end of each collection period (d22) blood samples were taken 4 hours after mineral supplement intake. The daily offered diets were composed of 5.5 kg hay, 0.2 kg soaked sugar beet pulp and 35 - 70 g complementary feed (inorganic trace element sources) admixed to the beet pulp, beside distilled water was offered ad libitum. Trace element intake in trial 1 and 2 is given in table 1. Mineral analyses of feed, faeces, urine and blood were performed. Regardless of the trace element intakes the macro-mineral contents of the whole diet (g/kg DM) were similar at Ca: 5.46 - 5.58; P: 2.66 - 2.86; Mg: 2.13 - 2.22. The diets were offered successively. Prior to each trial the ponies received conventional mineral supplement for a minimum of 20 days. Statistical analysis was performed by using the SAS® software (ANOVA mixed models).

Results: At a Cu-intake adjusted to the recommendations of the GfE (trial 2), the ponies showed a positive apparent Cu-digestibility and a positive Cu-balance, whereas the ponies needed more than 4 mg Zn/kg $BW^{0.75}$ to retain zinc. Intake of 0.01 mg Se/kg $BW^{0.75}$ resulted in a positive apparent Se-digestibility, but total Se-excretion (faeces and urine) was higher than the Se-intake (Table 1).

Table 1: Daily Cu-, Zn- and Se-intake (mg/kg BW 0.75), apparent digestibility (aD) and balance (bal) in trial 1 and 2

GfE 2014: recommendations (mg/kg $BW^{0.75}$): Cu:1, Zn: 4, Se: 0.01

Element	Cu			Zn			Se		
Trial	**Intake**	**aD (%)**	**bal (%)**	**Intake**	**aD (%)**	**bal (%)**	**Intake**	**aD (%)**	**bal (%)**
1	0.593	-7.28 [a]	-8.59 [a]	4.09	-11.0 [a]	-11.4 [a]	0.011	12.2 [a]	-6.50 [a]
	± 0.045	± 6.45	± 6.39	± 0.396	± 0.663	± 0.759	± 0.001	± 4.19	± 6.43
2	0.991	6.77 [b]	5.97 [b]	6.13	5.00 [a]	4.79 [b]	0.021	19.2 [a]	4.76 [a]
	± 0.065	± 3.70	± 4.07	± 0.535	± 4.10	± 4.11	± 0.001	± 5.69	± 3.72

Different letters indicate significant differences (p

The serum concentrations of the trace elements didn't show any directed changes. The average serum Zn- and Se-concentrations were slightly higher in trial 2 (Zn: 44.8 vs. 42.5 µg/dl, Se: 14.1 µg/dl vs. 12.9 µg Se/dl) whereas the Cu-concentrations were lower in trial 2 (93.0 µg Cu/dl vs. 116 µg Cu/dl).

Conclusion: At a roughage rich diet, that means at a sufficient dietary macro-mineral level (Ca/P/Mg: 2/1.5/3 times higher than the GfE 2014 recommendation), the recommended daily amount of 4 mg Zn/kg $BW^{0.75}$ (corresponds to 69.7 mg Zn/kg DM in this study) did not cover the needs of the ponies for maintenance. This raises the question whether the recommendation for zinc should be elevated. A daily intake of 1 mg Cu/kg $BW^{0.75}$ (here: 16.0 mg Cu/kg DM) and 0.01 mg Se/kg $BW^{0.75}$ (here: 0.175 mg Se/kg DM) resulted in positive apparent digestibilities. Both concentrations were adapted to the GfE 2014.

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58.

Effect of BioPlus® YC (*Bacillus subtilis* and *Bacillus licheniformis*) supplementation in diets with low- and high-protein content on intestinal microbiota composition of growing pigs

Wirkung von BioPlus® YC-Supplementierung (Bacillus subtilis und Bacillus licheniformis) in Rationen mit niedrigem oder hohem Proteingehalt auf die Zusammensetzung der intestinalen Mikrobiota beim Mastschwein

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Since the use of antimicrobial growth promoters was prohibited by the EU in 2006, probiotics seem to be an alternative for improving animals' health and performance. Within this regard, *Bacillus* spp. have the ability to sporulate, thereby making them stable during thermal treatment of feed and resistant to enzymatic digestion along the gastrointestinal tract. Thus, *Bacillus* spp. are frequently supplemented to pig diets, with BioPlus® YC being a commercial probiotic, containing *Bacillus subtilis (B. subtilis)* and *Bacillus licheniformis (B. licheniformis)* (Chr. Hansen A/S, Hørsholm, Denmark). Positive effects of dietary supplementation of *B. subtilis* and *B. licheniformis* on pigs' growth performance have been reported before (1), however, studies concerning the impact on pigs' intestinal microbiota composition are still lacking. Furthermore, there is increasing evidence that interactions of supplemental probiotics with dietary CP level might affect the intestinal microbiome at the ileal level. Therefore, the objective of this study was to determine the effect of BioPlus® YC supplemented to low- and high-protein diets on pigs' ileal gut microbiota composition.

Methods: Eight ileally cannulated pigs with an initial BW of 29 ± 1 kg were randomly allocated to a row-column design with 8 pigs and 3 periods of 15 d each. The assay diets were based on wheat-barley-soybean meal with 2 protein levels: low-protein (14% CP, as-fed; LP) and high-protein diet (18% CP, as-fed; HP). The LP diet was accomplished by blending the HP diet with 25% of native cornstarch. As a result, CP and AA levels in the LP diet amounted to 75% of HP diet, whereas contents of oil, minerals, vitamins and titanium dioxide were the same for all diets. The LP and HP diets were supplemented with or without BioPlus® YC (0.04%, as-fed). The pigs received the assay diets in meal form at a daily level of 4% (as-fed) of their average BW. The initial 14 d of every period were considered as adaptation period. Thereafter, ileal digesta samples were collected on d 15. Ileal gene copy numbers of bacteria were determined using quantitative real-time PCR. Data were analyzed as a 2 × 2 factorial design using the Proc Glimmix of SAS.

Results: The HP diet increased abundance of *Lactobacillus* spp. and *Bifidobacterium* spp. ($P < 0.05$). No effect of CP content on the ileal gene copy numbers of total bacteria, *Roseburia* spp., *Enterobacteriaceae*, *Bacteroides-Prevotella-Porphyromonas*, *Clostridium* cluster IV, *B. subtilis* and *B. licheniformis* was found. Likewise, no significant effect of supplementation of BioPlus® YC was observed for ileal gene copy numbers of total bacteria, *Lactobacillus* spp., *Bifidobacterium* spp., *Enterobacteriaceae*, *Clostridium* cluster IV, *B. subtilis* and *B. licheniformis*. However, dietary supplementation of BioPlus® YC increased ($P < 0.05$) abundance of *Roseburia* spp. and *Bacteroides-Prevotella-Porphyromonas*, while it tended ($P < 0.10$) to promote *B. subtilis, B. licheniformis* and total bacteria. Furthermore, there was an interaction ($P < 0.05$) of protein level and BioPlus® YC supplementation for ileal gene copy numbers of *Bacteroides-Prevotella-Porphyromonas*. The LP diet supplemented with the BioPlus® YC resulted in higher ($P < 0.05$) abundance of *Bacteroides-Prevotella-Porphyromonas* than the LP diet without BioPlus® YC supplementation, but did not differ from the HP diet.

Conclusions: Dietary supplementation with BioPlus® YC and differing dietary protein content affect porcine gut microbiota composition differently. Higher ileal gene copy numbers of *Roseburia* spp. following supplementation with BioPlus® YC may be beneficial due to ascribed health promoting properties of the butyrate producer. Furthermore, higher abundance of *Lactobacillus* spp. and *Bifidobacterium* spp. in the HP compared to the LP diet may positively affect gut health due to inhibition of pathogenic bacteria in the presence of lactic acid and increased bacteriocin production.

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59.

Effects of amylase and protease supplementation on rumen starch degradation in non-lactating Holstein cows fed a maize-based diet

Effekte einer Amylase und Protease Supplementierung auf den ruminalen Stärkeabbau bei einer maisbasierten Fütterung von trockenstehenden Holstein-Kühen

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Question: The efficiency of rumen fermentation is dependent on and limited by the chemical properties of the feed (e. g. plant cell wall digestibility, chemical composition of the grain). Feed additives may improve feed utilization in ruminants. For example, exogenous enzymes are administered to ruminant diets in order to support ruminal digestive capacity.

Methods: Eight non-lactating rumen-fistulated cows were used to study rumen dry matter and starch degradability according to the *in situ*-method supplemented with exogenous enzymes (amylase, protease) with following treatments: Control (Con): no enzyme addition; addition of amylase (A): 300 amylase-units (AU)/kg dry matter (DM); addition of protease (P): 15000 protease-units (PU)/kg DM; combination of both enzymes (AP): 150 AU + 7500 PU/kg DM (1 AU = the amount of enzyme that releases 6 µmol p-nitrophenol per minute from 1.86 mM ethylidene-G7-p-nitrophenyl-maltoheptaoside at pH 7.0 and 37 °C; 1 PU = the amount of serine protease that liberates 1 µmol para-nitroaniline (pNA) from 1mM Suc-Ala-Ala-Pro-Phe-pNA ($C_{30}H_{36}N_6O_9$) substrate per minute at pH 9.0 and 37 °C). The enzymes were mixed into the TMR directly before feeding. Cows were applied as a Latin square for four periods (double 4×4). During each period two cows received the same treatment. 7.0 kg DM of TMR were fed in two equal portions per animal and day consisting of 49% maize silage, 20% maize grain, 15% grass silage, 10% hay, and 6% soybean meal. Samples of the TMR and the single ingredients were weighed into nylon bags without previous drying or grinding. The enzymes were added to the content of the nylon bags in the same proportion as it was presented in the TMR to guarantee homogenous presence in all tested material. The nylon bags (4 replicates) were incubated in the rumen just before the morning feeding for 1, 2, 3, 4, 5, 6, 9, 12, 24, and 48 hours. Starch content of the bag residues was measured enzymatically by using a thermostable amylase (1) with following determination of glucose. Statistical analyses were carried out by 2-way ANOVA.

Results: The following table presents the starch disappearance of maize silage and maize grain and shows that the combination of both enzymes resulted in an increased starch disappearance of maize grain. The other treatments indicated no effects regarding the starch disappearance. SEM represents the standard error of means, different superscripts show significant differences between treatments ($p \leq 0.05$).

Feedstuff	Treatment	Starch disappearance (%)					
		1h	3h	6h	9h	12h	24h
Maize silage	Con	42.9	43.7	52.8	51.7	64.9	84.0
	AP	41.5	42.4	45.3	53.7	67.2	82.9
	SEM	5.0	3.6	5.5	3.4	3.5	2.4
Maize grain	Con	28.9	30.8	34.7^b	37.8^b	43.5^b	67.6^b
	AP	29.7	33.8	37.4^a	42.1^a	49.5^a	73.9^a
	SEM	1.6	1.2	0.8	1.0	1.4	2.1

Conclusion: The combination of both enzymes led to a consistently higher dry matter disappearance from 1 h up to 24 h and an increased effective dry matter disappearance from the rumen of both maize silage and maize grain (2). This effect is attributed to an increased ruminal starch degradability of maize grain due to the AP-treatment. Maize silage showed no measurable differences regarding the starch disappearance.

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60.

Effects of malate at different pH on ruminal fermentation in an artificial rumen (RUSITEC)

Effekte von Malat bei unterschiedlichen pH-Werten auf die ruminale Fermentation im künstlichen Pansen (RUSITEC)

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Dicarboxylic acids such as aspartate, fumarate, malate provide an effective alternative to improve production efficiency in ruminants. To our knowledge, however, there is no information about the effects of malate at different pH (7.0 and 5.5) on ruminal fermentation characteristics by rumen microbes. The objective of the present study, therefore, was to investigate the effects of malate on the *in vitro* fermentation of a 20 : 80 forage : concentrate diet when incubated under normal and subacute ruminal acidosis (SARA) conditions.

Methods: The study was carried out using the rumen simulation technique (RUSITEC) according to Czerkawski and Breckenridge (1). The RUSITEC system consisted of eight vessels with a capacity of 1 L each. Each vessel received daily 20 g of a basal diet consisting of 4 g barley straw and 16 g concentrate. The inoculum was obtained from a freshly slaughtered beef bull (500 kg mean body weight) at a commercial slaughter facility and transferred in warm (39 °C) insulated flasks to the *in vitro* system within 30 min. After an adaptation period of seven days, the fermentation parameters were determined for seven consecutive days. During this last period the eight vessels were divided into four groups. Two vessels received no additive (control) and two vessels received daily 500 mg DL-malate (Rumalato®) at pH 7.00. In the other two groups of the study the vessels were maintained at pH 5.5 by altering artificial saliva composition. Under acidosis condition two vessels received no additive (SARA) and two vessels received daily 500 mg DL-malate (Malate_SARA). Means were analyzed with two-way ANOVA and Duncan's post-hoc test. Significance was accepted at $P<0.05$.

Results: The effects of DL-malate on the *in vitro* rumen parameters under normal and acidosis conditions are given in the following table.

Item	Treatments				SEM	P-value
	Control	Malate	SARA	Malate_SARA		Time x Treatment
pH	6.80[a]	6.80[a]	5.77[b]	5.78[b]	0.01	<0.001
NH_3-N (mmol/l)	14.21[a]	14.71[a]	11.11[b]	11.50[b]	0.33	<0.001
Protozoa (x10^3)	4.45[a]	4.38[a]	1.88[b]	1.33[b]	351	0.08
DMD (%)	48.86[a]	48.43[a]	42.68[b]	43.92[b]	0.72	0.01

DMD: Dry matter digestibility

Conclusion: In the present study, rumen parameters were affected mainly by acidogenic conditions thus, NH_3-N concentration, protozoa number and DMD decreased dramatically depending on low ruminal pH (2). Malate, however, had no significant effects on ruminal fermentation in both conditions. Further *in vitro* and *in vivo* studies with different type of diets at different doses are required to determine the value of malate as feed additive to enhance the efficiency of ruminal fermentation, especially in low pH conditions.

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61.

Effects of propolis on ruminal fermentation in an artificial rumen (RUSITEC)

Effekte von Propolis auf die ruminale Fermentation im künstlichen Pansen (RUSITEC)

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Propolis is a resinous substance collected by honeybees from exudates and buds of the plants and mixed with wax and bee enzymes. It presents a wide range of biological and pharmacological properties, such as antibacterial, antiviral, anti-inflammatory, antifungal, antitumor, antioxidant, and hepatoprotective activities. Gonsales et. al. (1) evaluated the antibacterial activity of 22 propolis samples collected in Brazil against two bacterial strains using agar disc diffusion method. Accordingly, they recorded that the antibacterial activity of propolis was higher against gram-positive bacteria relative to gram-negative bacteria, showing a positive correlation with flavonoids content. Therefore, we hypothesized that propolis might be a possible alternative to the use of antibiotics as feed additives in animal production. The aim of this study was to investigate the effects of propolis on the *in vitro* fermentation of a 40 : 60 forage : concentrate diet using the rumen simulation technique (RUSITEC).

Methods: The study was carried out using the rumen simulation technique (RUSITEC) according to Czerkawski and Breckenridge (2). The complete unit consisted of six fermentation vessels with a capacity of 1 L each. Each vessel received daily 10 g of a basal diet consisting of 4 g pelleted alfalfa hay and 6 g pelleted concentrate. Rumen content was obtained from a pooled sample from two freshly slaughtered mature Merino sheep and transferred to the *in vitro* system within 30 min. After an adaptation period of seven days, the fermentation parameters were determined for seven consecutive days. During this last period the six fermentation vessels were divided into 3 groups with two vessels per group. The first group served as control and received daily 0.5 ml of 70% ethyl alcohol in water (without having any propolis). The second and third groups received daily 0.5 ml of 20% (contained active substances of 100 mg crude propolis) or 60% ethanolic extracts of propolis (contained active substances of 300 mg crude propolis), respectively. Data were analyzed using one-way repeated measures ANOVA followed by Duncan's-test.

Results: The effects of different concentrations of ethanolic extract of propolis (EEP) on the *in vitro* rumen parameters are given in the following table.

Variable	Control	20% EEP (0.5 ml/day)	60% EEP (0.5 ml/day)
pH	6.80 ± 0.01	6.81 ± 0.01	6.80 ± 0.02
Total SCFA (mmol/day)	37.87 ± 1.51	38.13 ± 1.97	37.23 ± 1.84
Acetate	23.83 ± 0.94	23.78 ± 1.72	23.51 ± 1.25
Propionate	11.15 ± 0.79^{a}	11.18 ± 0.59^{a}	10.59 ± 0.79^{b}
Butyrate	2.89 ± 0.25^{a}	3.17 ± 0.29^{b}	3.13 ± 0.34^{b}
Acetate : propionate ratio	2.14 ± 0.14	2.13 ± 0.18	2.23 ± 0.16
Total bacteria (x 10^{8}/mL)	16.35 ± 0.70^{a}	15.47 ± 1.18^{b}	14.84 ± 1.30^{b}
Total protozoa (x 10^{3}/mL)	2.81 ± 0.32	2.90 ± 0.31	3.04 ± 0.41
NH_3-N (mmol/L)	9.51 ± 0.35^{a}	7.25 ± 0.57^{b}	5.78 ± 0.28^{c}
Dry matter digestibility (%)	62.93 ± 2.53	62.43 ± 2.10	61.21 ± 3.47

$^{a\text{-}c}$Means in the same row followed by different superscripts differ (P

Conclusion: The present study showed that propolis at assayed doses did not improve the production rate and the profile of ruminal SCFA and it would not be nutritionally beneficial to the ruminal energetic metabolism. However, propolis was able to inhibit ruminal NH_3-N concentration. This ammonia-reducing effect may help to improve the nitrogen retention in ruminants.

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62.

Effects of chronic feeding of quercetin on growth performance of rainbow trout and common carp

Einfluss einer chronischen Fütterung von Quercetin auf die Wachstumsleistung von Regenbogenforelle und Karpfen

Ansari F., *Blank R., Wolffram S. – Kiel

The flavonol quercetin is one of the most abundant flavonoids in plants and also one of the most studied in humans and animals. Quercetin has numerous health-promoting activities such as antiviral, antibacterial, anticarcinogenic and anti-inflammatory effects. Many of these beneficial properties of quercetin are probably due to the antioxidant properties of quercetin. The aim of this study was to investigate the effect of dietary quercetin supplementation on growth performance of carnivorous rainbow trout *(Oncorhynchus mykiss)* and omnivorous carp (*Cyprinus carpio*).

Materials and Methods: Experiments were performed separately for each species in the experimental facilities of the Gesellschaft für Marine Aquakultur mbH (Büsum, Germany). Rainbow trouts or carps with a body weight of 164.7 ± 3.9 and 176.8 ± 3.1 g, respectively, were housed in 20 tanks (150 L, 10 fish per tank) with 5 replications for each dietary treatment. Dietary treatments consisted of a flavonoid free control diet supplemented with 0, 2.5, 5.0, and 7.0 g/kg quercetin aglycone (Roth, Germany). All diets were manufactured by Altromin Spezialfutter GmbH & Co. KG, Lange, Germany. After an acclimatization period of 2 weeks (control diet), fish were fed their respective experimental diets by hand to apparent satiation twice per day (9.00 and 17.00 h) for 6 (trout) and 5 (carp) weeks. Fish were weighted every 2 weeks, and daily feed intake was recorded. Data were statistically analysed according to a one-factorial repeated measure design using Proc mixed (SAS). Comparisons of means were performed using the Tukey Test.

Results: Feeding increasing doses of quercetin had no effect on feed intake, daily gain and final body weight in rainbow trout and carp (Table 1). However, in carp the highest dose of quercetin improved feed conversion significantly.

	Quercetin, g/kg					
	0	2.5	5.0	7.0	SEM	P-Value
Rainbow trout						
Initial weight, g	160.8	161.6	161.5	161.5		
Final weight, g	283.2	291.6	268.9	277.3	5.65	0.69
Feed intake, g/d	3.9	3.4	3.6	3.4	0.29	0.53
Daily gain, g	2.9	2.9	2.6	2.8	0.26	0.74
Feed conversion, g/g	1.43	1.18	1.44	1.27	0.15	0.56
Specific growth rate, %/d	1.34	1.40	1.21	1.28	0.08	0.38
Carp						
Initial weight, g	176. 0	176.8	176.7	176.7		
Final weight, g	390.5	396.9	397.8	405.2	3.17	0.14
Feed intake, g/d	9.1	9.1	8.8	8.8	0.13	0.22
Daily gain, g	6.1	6.1	6.3	6.5	0.14	0.13
Feed conversion, g/g	1.49^{a}	1.48^{a}	1.40^{ab}	1.34^{b}	0.03	0.02
Specific growth rate, %/d	2.26	2.31	2.32	2.37	0.03	0.13

^{a,b} Values within a row bearing no common superscript are significantly different ($P<0.05$; n=10)

Conclusions: The present results indicate, that dietary quercetin supplementation, at least in the doses administered in these studies, does not have any noteworthy effect on growth performance of rainbow trout or carp.

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63.

Effects of chronic feeding of quercetin on antioxidant enzyme systems of rainbow trout and common carp

Einfluss einer chronischen Fütterung von Quercetin auf antioxidative Enzymsysteme bei Regenbogenforelle und Karpfen

Ansari F., *Blank R., Wolffram S. – Kiel

The flavonol quercetin is one of the most abundant flavonoids in plants and also one of the most studied in humans and animals. Quercetin has numerous biological activities such as antiviral, antibacterial, anticarcinogenic and anti-inflammatory effects. Quercetin is one of the most potent antioxidants among polyphenols and thus could play a health-promoting role not only in living fish but also for storage stability of consumable fish products. Thus the aim of the present study was to investigate the effect of chronic feeding of various doses of quercetin on the activity of antioxidant enzyme systems, like catalase (CAT) and copper-zinc superoxide dismutase (SOD) in liver and muscle of omnivorous carp (*Cyprinus carpio*) and carnivorous rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods: Experiments were performed separately for each species in the experimental facilities of the Gesellschaft für Marine Aquakultur mbH (Büsum, Germany). Rainbow trouts or carps with a body weight of 164.7 ± 3.9 and 176.8 ± 3.1 g, respectively, were housed in 20 tanks (150 L, 10 fish per tank) with 5 replicates for each dietary treatment. Dietary treatments consisted of a flavonoid free control diet supplemented with 0, 2.5, 5.0, and 7.0 g/kg quercetin aglycone (Roth, Germany). All diets were manufactured by Altromin Spezialfutter GmbH & Co. KG, Lange, Germany. After an acclimatization period of 2 weeks (control diet), fish were fed their respective experimental diets by hand to apparent satiation twice per day (9.00 and 17.00 h) for 6 (trout) and 5 (carp) weeks. At the end of experiments, 2 fishes from each tank were removed, liver and muscle samples were taken and stored at -70 °C until analysis. The activities of SOD (19160 SOD Assay Kit-WST) and CAT (CAT100) were measured using commercial kits (Sigma-Aldrich Biochemie GmbH, Hamburg, Germany). Data were statistically analysed according to a one-factorial repeated measure design using Proc mixed (SAS). Comparisons of means were performed using the Tukey Test.

Results: Catalase activity could not be detected in the muscle tissue of trout and carp. Activity of catalase in the liver of trout and carp was not affected by increasing quercetin supplementation in the diet. SOD activity in muscle of trout and in liver of carp showed a nonlinear (quadratic) dose response with the highest activity ($P < 0.05$) at a quercetin concentration of 5 g/kg diet, whereas, SOD activity in liver of trout and in muscle of carp was not affected by quercetin.

	Quercetin; g/kg diet				
	0	2.5	5.0	7.0	SEM
Rainbow trout					
Liver CAT (µmol/min/mg protein)	1622.4	1899.1	1545.3	1722.6	151.71
Liver SOD (U/mg protein)	260.5	254.9	261.4	266.4	12.07
Muscle SOD (U/mg protein)	17.5^{a}	22.5^{bc}	26.1^{c}	19.0^{ab}	1.61
Carp					
Liver CAT (µmol/min/mg protein)	1152.0	1016.3	1044.1	863.5	88.37
Liver SOD (U/mg protein)	103.8^{a}	115.2^{a}	176.6^{b}	125.5^{ab}	18.40
Muscle SOD (U/mg protein)	36.5	40.3	40.5	40.4	1.75

^{a,b,c}Values within a row bearing no common superscript are significantly different (P

Conclusions: The results showed that quercetin supplementation has no pronounced effect on antioxidative enzyme systems like SOD and CAT in rainbow trout and carp. However, the dose dependent increase in SOD activity in liver and muscle of trout and carp could be interpreted as a mild pro-oxidative effect of quercetin.

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64.

Effects of chronic feeding of quercetin on body composition of rainbow trout and common carp

Einfluss einer chronischen Fütterung von Quercetin auf die Körperzusammensetzung von Regenbogenforelle und Karpfen

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The flavonol quercetin is one of the most abundant flavonoids in plants and also one of the most studied in humans and animals. Quercetin has antiviral, antibacterial, anticarcinogenic and anti-inflammatory effects. Many of these beneficial properties of quercetin are probably due to the antioxidant properties of quercetin. Furthermore, quercetin reduced fat accumulation in liver and white adipose tissue in mice possibly by regulating lipid metabolism genes (1) and decreased crude lipid content in Nile tilapia after feeding quercetin for a period of 4 weeks (2). Thus, the aim of this study was to investigate the effect of chronic feeding of quercetin on the body composition of rainbow trout *(Oncorhynchus mykiss)* and common carp *(Cyprinus carpio)*.

Materials and Methods: Experiments were performed separately for each species in the experimental facilities of the Gesellschaft für Marine Aquakultur mbH (Büsum, Germany). Rainbow trout or carp with a body weight of 164.7 ± 3.9 and 176.8 ± 3.1 g, respectively, were housed in 20 tanks (150 L, 10 fish per tank) with 5 replications for each dietary treatment. Dietary treatments consisted of a flavonoid free control diet supplemented with 0, 2.5, 5.0 and 7.0 g/kg quercetin aglycone. All diets were manufactured by Altromin Spezialfutter GmbH & Co. KG, Lange, Germany. After an acclimatization period of 2 weeks (control diet), fish were fed their respective experimental diets by hand to apparent satiation twice per day (9.00 and 17.00 h) for 6 (trout) and 5 (carp) weeks. Prior to the start of the experiments and at the end, 2 fishes from each tank were removed, freeze-dried for 120 h and homogenized. Analysis of body composition was performed according to the methods outlined by theVDLUFA. Data were statistically analysed according to a one-factorial repeated measure design using Proc mixed (SAS). Comparisons of means were performed using the Tukey Test.

Results: At the end of the feeding experiments, no effect of quercetin supplementation on dry matter, crude protein, crude lipid, ether extract, ash, or energy content were detected.

	Quercetin, g/kg					
	0	2.5	5.0	7.0	SEM	P-value
Rainbow trout						
Dry matter,%	29.5	28.9	29.0	29.1	0.65	0.983
Crude protein, %	54.5	53.6	53.2	53.3	0.92	0.401
Ether extract, %	33.6	33.3	36.2	37.1	1.48	0.282
Ash, %	7.3	7.2	7.2	7.3	0.22	0.953
Energy, MJ	26.6	26.7	26.8	26.7	0.26	0.412
Carp						
Dry matter, %	25.4	25.1	25.3	25.4	0.48	0.793
Crude protein, %	57.2	56.7	56.4	55.9	1.22	0.856
Ether extract, %	28.3	29.2	28.8	29.7	1.58	0.468
Ash, %	11.1	11	10.5	11.1	0.39	0.445
Energy, MJ	24.5	24.4	24.8	24.4	0.38	0.577

Conclusions: The present results indicate, that chronic quercetin supplementation, at least in the doses administered in these studies, has no effect on body composition of rainbow trout and carp.

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65.

Influence of added oregano oil in wheat based diets on laying performance and microbial parameters of excreta

Einfluss von Oregano-Öl in weizenbetonten Futtermischungen auf Leistungsparameter und mikrobiologischen Status der Exkremente von Legehennen

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Several *in vitro* and *in vivo* studies with growing chicken and piglets indicated antimicrobial and antioxidative effects of essential oils resulting in increased performance parameters [1]. However, a corn based diet supplemented with oregano oil did not yield any effect both on performance parameters of laying hens and egg quality [2]. The application of wheat based diets could be limited by the level of non-starch polysaccharides resulting in reduced nutrient digestibility and modified intestinal microflora [1]. The current study was conducted to evaluate the effects of added oregano oil in wheat based diets for laying hens under suboptimal conditions (heat treated wheat and wheat bran, accumulation of excreta in the environment) on laying performance and microbial status of excreta.

Methods: 72 Lohmann Brown-Classic hens at 26th week of age were divided into 2 groups (A: control diet; B: diet with 500 g/t oregano premixed powder) with 3 boxes per group and 12 birds per box for a period of 12 weeks. The basal diet contained 45 % wheat and 22.5 % wheat bran. The wheat/wheat bran mixture was hydrothermal processed under extreme processing conditions (addition of 2 % saturated steam, 130 °C, 20 bar, 75 kWh/t energy input) to achieve an increased solubility of the pentosan fraction. To intensify the microbial pressure, during 12 weeks of the trial the litter (straw) was not exchanged or renewed.

At the beginning and at the end of the trial, excreta of 18 hens per group were studied for presence or counts of several microbes and parasites (*Campylobacter* spp., *Salmonella* spp., aerotolerant clostridia, *E. coli*, *Staphylococcus* spp. total, koagulase positive and negative, coccidian, histomonas).

Feed intake (FI), body mass (BM), laying performance (LP), egg mass production (EMP) and feed conversion ratio (FCR) were measured weekly. Experimental data were submitted to ANOVA (IBM SPSS Statistics, vers. 23).

Results: The addition of oregano oil in layer feed provided no significant effect ($p > 0.05$) on performance parameters (Tab. 1). Only a numerical increase of FI and EMP was observed but cannot be pointed out with practical relevance.

Table 1: Cumulative performance parameters of laying hens (means ± sd)

Group	LM [g]	FI [g]	LP [%]	EMP [g]	FCR [g feed/g EMP]
A	1971 ± 66	118.8 ± 2.4	96.6 ± 2.6	60.5 ± 3.9	2.00 ± 0.11
B	1967 ± 59	120.1 ± 1.3	98.0 ± 1.4	61.9 ± 4.2	1.98 ± 0.09

As well as at beginning also at the end of the study campylobacter, salmonella, clostridia and coccidian were below limit of detection. Only in one case of control group the test on histomona was positive at the end of the trial. The content of *E. coli* was mathematical significantly increased during the trial from 1.88 x 10^8 to 4.41 x 10^8 CFU/g excreta ($p = 0.0004$), but without practical relevance as well as without significant differences between the both groups (A: 4.72 x 10^8 CFU/g excreta, B: 4.11 x 10^8 CFU/g excreta, $p = 0.614$).

Conclusions: In spite of the aimed suboptimal feeding and housing conditions, the laying hens yielded optimal performance data. Consequently, under these circumstances added oregano oil did not act on zootechnical data. Accordingly, an effect on microbes and parasites was not observed.

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66.

Influence of a yeast cell wall product on immunological traits of broiler chickens

Effekte der Zulage eines Hefezellwandproduktes auf immunologische Parameter bei Broilern

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Yeast cell walls can influence the immune response of animals. However, results of studies are inconsistent and seem to be dependent on the yeast sources, processes and composition of products. Products containing *Saccharomyces cerevisiae* affected the immune response in challenge trials with *Eimeria* spp. (1). Mannanoligosaccharides and β-glucans act as immunomodulators affecting the innate (2) and the acquired immune system of chickens (3) and seem to be detected as antigens by the gut-associated immune system. A dose dependent relation between dietary yeast cell wall products and the immune response is not yet clear. Therefore, the study was conducted to investigate whether a yeast cell wall product can have concentration-dependent effects on immunological traits in broiler chickens.

Methods: One day-old male broiler chickens (Cobb Germany Avimex GmbH) were used in two consecutive trial runs and samples were taken during as well as at the end of the 35-day-lasting fattening period for immunological analysis. The starter (d 1 - 14) and grower diets (d 15 - 35) were based on soybean meal, maize and wheat. Four experimental groups were fed the product (ImmunoWall®, MIAVIT GmbH, Essen-Oldenburg) in concentrations of 0.05 %, 0.10 %, 0.20 % and 0.30 % over the whole fattening period, while the fifth experimental group served as control; eight birds were sampled in each group. Thymus, spleen and *bursa fabricii* were removed and weighed to determine the relative organ weights at d 35. On day 21 the animals were vaccinated against NDV and blood samples were taken directly before as well as seven and 13 days after the vaccination for the quantification of the antibody titres against NDV, the phagocytic activity of blood monocytes and for complete blood counts. Digesta from the duodenum were collected to measure the intestinal IgA concentration. All collected data were analysed by using a one-factorial analysis of variance or a Kruskal-Wallis-test as well as a polynomial contrast analysis ($p<0.05$).

Results: Overall performance of the birds was not affected. The relative immune organ weights, the intestinal IgA concentration, the phagocytic activity of the monocytes, the antibody titres against NDV as well as the concentrations of monocytes and heterophils in the blood of the broiler chickens were not affected by the addition of the yeast cell wall product, whereas the concentrations of the lymphocytes in the blood were numerically higher at all three collection time points. Furthermore, the lymphocytes and in tendency the T- and B-lymphocytes in the blood samples were higher on day 34 in the experimental groups with the addition of the yeast cell wall product (table 1).

Table 1: Results of the blood count [cell counts x 10^3/µl blood] of the five experimental groups on day 34 (median (minimum - maximum); n = 7 - 8

	Yeast cell wall product					**p**
	0 %	0.05 %	0.10 %	0.20 %	0.30 %	
Lymphocytes	10.8 (8.7 -13.7)^{b}	14.7 (10.2 - 16.6)^{a}	12.8 (9.5 - 16.4)^{ab}	13.9 (11.2 - 20.2)^{a}	13.1 (10.9 - 14.8)^{ab}	0.037
T-cells	9.2 (7.9 - 11.8)	12.1 (8.7 - 14.2)	11.1 (7.8 - 12.9)	11.4 (9.4 - 17.2)	11.4 (9.1 - 12.9)	0.084
B-cells	1.4 (0.8 - 2.1)	2.5 (0.6 - 2.8)	2.0 (1.0 - 3.5)	2.4 (1.6 - 3.3)	1.9 (1.6 - 2.1)	0.066

Means in the same row with different superscripts differ significantly ($p < 0.05$; Mann-Whitney-test).

Conclusion: The results indicate that the addition of the yeast cell wall product had an impact on selected traits of the adaptive immune system of broiler chicken. It should be clarified, how far the addition of the yeast cell wall product can be used in chicken under less favourable housing conditions in order to improve the health and performance of the animals.

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67.

Variation of carbohydrate content and degree of polymerization along the equine gastrointestinal tract

Variabilität des Kohlenhydratgehaltes und des Polymerisationsgrades entlang des Verdauungstraktes von Pferden

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Prebiotic doses of inulin (INU) and fructooligosaccharides (FOS) are known to stimulate hindgut microbiota in horses despite their partial degradation in the stomach (1). We hypothesized that a combination of FOS+INU would substantially increase the concentration of fructans in the hindgut of horses while a possible degradation in the stomach would be reduced. The aim of the study was to investigate whether long-term supply of prebiotic doses of FOS+INU via Jerusalem artichoke meal (JAM) elevates fructan content in the large intestine.

Methods: During 3 weeks, 2 x 6 healthy adult warmblood horses (10 mares, 1 stallion, 1 gelding; body weight [BW] 534 ± 64.5 kg) were fed crushed oat grains (1 g starch/kg BW d^{-1}) and meadow hay (1.5 kg/100 kg BW d^{-1}) in 2 equal meals/d. Additionally they received either 0.2 g FOS+INU/kg BW d^{-1} *via* JAM or an equal amount of a placebo (maize cob meal without grains, CON). At the end of the experiment horses were euthanized approximately 1 h after they received ½ of the daily ration. Digesta samples were collected from the stomach (pars nonglandularis, PN; pars glandularis, PG), caecum and colon (ventrale, dorsale and transversum) and analyzed for dry matter (DM), easily soluble carbohydrates (HPLC), starch (enzymatically) and the degree of polymerization (DP; *via* HPLC) of the fructans. Data were analyzed by two-way ANOVA (SPSS Version 21.0) at a significance level of $P<0.05$.

Results: Mono- and dimeric sugars and fructans reached detectable quantities in the stomach only (tab 1). Starch in the stomach amounted to ~ 178 g/kg DM in the PN and ~ 130 g/kg DM in the PG for both groups (tab 1) and in the intestine the concentrations were below 10 g/kg DM. Fructose, sucrose and fructans tended to be higher in JAM *vs* CON, for glucose the opposite was measured ($P > 0.05$). The concentrations of glucose, fructose, fructans and starch decreased ($P > 0.05$) along the digestive tract from PN towards PG, irrespective of the diet. In the PN, the fructans with DP up to 5 were higher in CON *vs* JAM, but in PG the fructans with DP up to 5 were higher in JAM as compared to CON. For fructans with DP 6-10, the opposite was true ($P > 0.05$).

Table 1: Content of carbohydrates in the diet as well as in the stomach [g/kg DM]

		glucose	fructose	sucrose	fructan	starch
hay		29.9	38.6	6.3	39.9	0
concentrate	CON	0.6	0.7	11.3	1.0	427
	JAM	1.0	4.6	19.0	33.9	427
digesta PN	CON	16.7	14.8	8.1	47.4	177
	JAM	13.8	15.4	7.1	61.8	178
digesta PG	CON	12.8	10.6	4.2	23.3	126
	JAM	9.2	12.1	9.9	43.0	137
pooled SD		0.73	0.87	1.46	4.34	7.41

Conclusion: The results indicate that mono- and dimeric sugars, fructans and starch from hay-based diets with minor supply of oat grains were almost completely decomposed already in the stomach of horses, and the prebiotic doses of FOS+INU elevated fructan concentration within the stomach. This leads to the conclusions that i) the supply of prebiotic substances according to recommendations is inadequate to stimulate hindgut microbiota and ii) fermentation of nonstructural carbohydrates might pose a risk for the stomachs´ health.

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68.

Impact of high dietary zinc concentrations on the horizontal antibiotic resistance gene transfer between enterobacteria in the intestine of gnotobiotic mice

Einfluss hoher Zinkkonzentrationen im Futter auf den horizontalen Transfer von Antibiotikaresistenzgenen in Darm gnotobiotischer Mäuse

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High concentrations of dietary zinc are widely used in piglets to reduce weaning-associated diarrhea and improve animal performance. However, a strong increase of intestinal *Escherichia coli* clones with multiple antibiotic resistances has been observed when piglets were fed a diet containing zinc at 2,500 ppm and it was proposed that zinc promotes horizontal gene transfer between gut bacteria (1). We aimed with our study to clarify in a highly-controlled gnotobiotic mouse model whether high concentrations of dietary zinc influence transfer of antibiotic resistance genes in the intestine.

Methods: Germ-free mice were fed a piglet diet based on wheat, barley, corn and soybean meal with zinc oxide at either 100 ppm or 1,900 ppm (atomic absorption spectrometry) and colonized with 10^8 cells of *E. coli* and *Klebsiella pneumoniae*, each. The latter strain served as a donor of an extended-spectrum β-lactamase-coding plasmid. Fecal material was collected every day over an experimental period of 20 days and colony forming units (cfu, $\log_{10}$) of *K. pneumoniae* (donor strain) and of *E. coli* without (recipient strain) and with (transconjugants) acquired antibiotic resistances were determined. Conjugation rates were calculated by dividing cfu of the transconjugant by cfu of the recipient strain. The area under the curve (AUC) approach was applied to integrate data obtained for each mouse, strain and diet group over the range of sampling time points. AUC values between groups were compared by Mann-Whitney U tests.

Results: The bacterial strains successfully colonized the intestine of previously germ-free mice. Fecal bacterial numbers were highly variable. The minimal and maximal $\log_{10}$ of colony forming units and the integrated AUC values are given in table 1. Median values for the animals on the low zinc diet were $\log_{10}$ 9.9 (donor strain), $\log_{10}$ 8.7 (recipient strain) and $\log_{10}$ 6.0 (transconjugant). When the high zinc diet was fed, median values were $\log_{10}$ 10.0 (donor strain), $\log_{10}$ 8.5 (recipient strain) and $\log_{10}$ 5.6 (transconjugant). No effect of diet was observed when the AUC of the donor and recipient values were compared. In contrast transconjugant AUC was significantly higher for the mice fed with 100 ppm of zinc ($P = 0.025$) indicating that zinc may inhibit the horizontal gene transfer. However, this assumption was not supported by the conjugation rate which did not differ between the feeding groups (data not shown).

Table 1: Colony forming units (log10) and AUC values of *K. pneumoniae* (donor) and of *E. coli* without (recipient) and with acquired antibiotic resistances (transconjugant) in mouse feces

Strain	**low zinc** minimal-maximal	**high zinc** minimal-maximal	**low zinc** median AUC	**high zinc** median AUC	**p values**
Donor	9.3 - 10.3	9.4 - 10.3	119.1	119.6	0.482
Recipient	6.4 - 9.6	7.3 - 9.6	104	101.9	0.225
Transconjugant	4.0 - 7.4	4.3 - 7.2	71.6	67.1	0.025

Conclusion: Our data does not provide a clear picture on possible effects of high dietary zinc on horizontal transfer of antibiotic resistance genes between intestinal bacteria because a significantly lower abundance of transconjugants was not supported by lower conjugation rates. The molecular mechanisms underlying the previously reported higher abundance of multi-resistant *E. coli* in piglets on a high-zinc diet require further investigation.

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69.

Probiotic supplementation with *Enterococcus faecium* NCIMB 10415 modulates the effects of an enterotoxigenic *Escherichia coli* strain on intestinal barrier function and TNF-α expression

Auswirkungen einer probiotischen Supplementierung mit Enterococcus faecium auf die Effekte eines enterotoxischen Escherichia coli-Stammes auf intestinale Barrierefunktionen und die Expression von TNF-α

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Postweaning diarrhea in piglets is often associated with an infection with enterotoxigenic *Escherichia coli* (ETEC). Supplementation with probiotic strains has been used to improve the health of piglets in the post-weaning period (1). Effects on barrier properties in the gastro-intestinal tract and the gut-associated immune system have been observed (2). For example, the proinflammatory cytokine release was decreased whereas the anti-inflammatory response was enhanced in a model of colitis (3). The aim of the present study was to investigate the effects of *Enterococcus faecium* NCIMB 10415 (*E. faecium*) on intestinal epithelial cells of porcine jejunum during a challenge with ETEC *in vitro*.

Methods: Porcine intestinal epithelial cells from piglet jejunum (IPEC-J2) were incubated with *E. faecium* or an ETEC strain or a combination of both strains in cell culture inserts or Ussing chambers. In case of coincubation, the cells were preincubated with the probiotic strain before the pathogenic strain was added. Transepithelial electrical resistance (R_t) was measured by a voltohmmeter in cell culture inserts. Furthermore, in cell monolayers mounted in Ussing chambers, R_t and transepithelial potential difference (PD_t) were continuously recorded under open circuit conditions. Samples for analysis of cytokine mRNA expression (Tumor necrosis factor alpha, TNF-α) were taken from 24-well plates and analysed by quantitative real-time RT-PCR. Furthermore the release of TNF-α in the supernatant was measured by ELISA. Statistical evaluation of the data was performed by variance analysis with the fixed factor 'treatment' ('control', '*E. faecium*', 'ETEC' and '*E. faecium* + ETEC'), and a post-hoc Scheffe test was conducted per time point.

Results: ETEC induced a decrease in R_t in epithelial cells compared to bacteria-free control cell monolayers or cells incubated with the probiotic ($p \leq 0.05$). Coincubation with *E. faecium* prevented this decrease in R_t at 2 and 4 h after ETEC addition ($p \leq 0.05$). In Ussing chambers, ETEC also induced a decrease in R_t. The cell monolayers incubated with the probiotic strain did not differ from the control but modulating effects of the coincubation could not be observed.

The mRNA expression of the proinflammatory cytokine TNF-α was significantly increased 4 h after incubation with the ETEC strain compared to the control and the *E. faecium* treated cells ($p \leq 0.05$). This increase was significantly reduced when cells were coincubated with the probiotic. On the protein level, the ETEC strain also increased the release of TNF-α compared to the control and the *E. faecium*-treated cells. However, the coincubated cells did not differ from the cells incubated with ETEC alone.

Conclusion: These results indicate a protective effect of the probiotic *E. faecium* when cells are submitted to a pathogenic challenge with ETEC.

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70.

Comparison between an *in vitro* method (Rostock fermentation test) and glass jar model silages to predict forage ensilability

Vergleich einer in vitro Methode (Rostocker Fermentationstest) mit Weckglassilagen zur Vorhersage der Silierbarkeit von Grünfutter

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Improving the conservation process with regard to maintaining the nutritional value of forages is a major task in animal nutrition. The ensilability of various plant materials and silage additives is commonly studied by glass jar model silages (GLASS), which is the method of choice on a national level, but rather laborious and time-consuming. The Rostock fermentation test (RFT) is a rapid method to forecast ensilability. We hypothesized that the RFT is suitable to predict the success of ensiling and thus the assessment from both RFT and GLASS is similar. The aim of the study was therefore to compare the predictive power of RFT using GLASS as basis of comparison.

Methods: GLASS and RFT were performed with plant material of good (maize), medium (fresh and wilted perennial ryegrass) and poor (fresh and wilted lucerne) ensilability. For RFT, 50 g minced plant material was mixed in beakers (600 ml) with 200 ml of a defined KCl solution (2.3, 1.5, 4.6, 2.1 and 8.4% for maize, fresh and wilted ryegrass and fresh and wilted lucerne, resp.) to set equal osmotic conditions as in GLASS (1.5 L capacity). Beakers of RFT were covered (aluminium foil) and incubated (30 °C). Treatments (n = 4) of RFT and GLASS were as follows: control without additive (CON), sucrose (2%, SUC), *Lb. plantarum* (DSM 8862 and 8866, $3x10^5$ cfu/g, LAB_1), LAB_1+SUC, *Lb. buchneri* (LN 4637/ATCC PTA-2494, $1x10^5$ cfu/g, LAB_2), LAB_2+SUC. The pH was determined in GLASS at 1.5, 3, 10 and 90 d and in RFT at 14, 18, 22, 26, 38 and 46 h, whereas lactic acid (HPLC) and volatile fatty acids and ethanol (GC) were determined at 3 and 90 d (GLASS) and at 46 h (RFT). Data were subjected to ANOVA (SPSS 19.0) followed by SNK test. A linear regression analysis was used to correlate pH and fermentation products from RFT and GLASS.

Results: In both GLASS and RFT the plant material influenced the pH value ($P < 0.001$), whereas pH over all treatments was always lowest in maize, followed by fresh and wilted ryegrass and highest in fresh and wilted lucerne. A ranking of treatments over all plant materials and storage durations (GLASS) respectively measurement times (RFT) showed for both methods the same order of efficacy ($P < 0.05$) regarding the lowest pH value achieved: LAB_1+SUC < LAB_1 < LAB_2+SUC < SUC < LAB_2 < CON, with exception that LAB_1 and LAB_2+SUC in RFT did not differ ($P > 0.05$). Unexpectedly, regarding single plant materials and treatments, only in fresh and wilted lucerne LAB_1 did not show an effect on pH reduction compared to CON ($P > 0.05$) until 22 h in RFT, but already at 1.5 d in GLASS, which needs to be clarified in further experiments. Comparing pH values of GLASS and RFT by means of linear regression reveals the closest relation between 10 d-silages and the 38 h-measurement ($R^2 = 0.81$; ±s = 0.21). Lactic acid was the primary acid in both methods with ratios of lactic acid to total organic acids ranging from 0.63 to 0.95, although concentrations of lactic acid were always higher in RFT than in GLASS, presumably due to the higher water availability in the aqueous suspension. The correlation between GLASS and RFT for lactic acid and acetic acid, ethanol and the ratio of lactic acid to total organic acids was only moderate, but generally better when 3 d instead of 90 d silages were compared with the 46 h measurement of RFT (e.g. $R^2 = 0.513$, ±s = 37.2 comparing 3 d and 46 h and $R^2 = 0.22$, ±s = 42.5 comparing 90 d and 46 h for lactic acid [g/kg dry matter]).

Conclusions: The correlations of pH value and fermentation parameters suggest that RFT better reflects the initial phase of silage fermentation than the long-term. This is in line with the absence of 1,2-propanediol in RFT, which is usually metabolized by *Lb. buchneri* from lactic acid, but only in the second phase of fermentation. However, with regard to a selection of fermentation stimulants (e.g. sugar or inoculants) to be applied to the plant material for ensilage, RFT will lead to the same conclusions as GLASS. Although some aspects of silage quality evaluation cannot be covered by RFT, such as determination of dry matter losses, aerobic stability or changes in structural carbohydrates, the *in vitro* method offers several advantages. Results of ensilability are obtained within a short time and as only a small amount of plant material is needed, a multitude of treatments can be tested within one RFT run.

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71.

Effects of a clinoptilolite supplementation on rumen fermentation *in vitro*

Wirkungen einer Klinoptilolith-Zulage auf die Pansenfermentation des Rindes in vitro

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Natural zeolites, negatively charged tectosilicates whose framework structures form channels and cages, feature molecular sieving and cation binding/exchanging properties. As feed additives for cattle, zeolites have shown to reduce the incidence and severity of diarrhea in calves, to increase the blood antibody level following colostrum administration, and to be an appropriate means to lessen calcium uptake in cows ante partum. Clinoptilolites, natural zeolites, have a high adsorption affinity for ammonia and are fed to ameliorate the health status and performance of animals. Such positive alterations could be induced by modifications in rumen fermentation. Therefore, the aim of this study was to identify a possible influence on the fermentation in the liquid ruminal phase by added clinoptilolite, particularly since the existing data regarding the effects of zeolites on rumen fermentation is inconsistent.

Methods: Using an artificial rumen (1), ruminal fluid was incubated with and without a ground clinoptilolite at two different levels for six hours. As carbohydrate and nitrogen sources, glucose and urea were supplied. Gas production was recorded and gaseous and liquid samples were obtained immediately after filling the incubators, after three and after six hours of incubation. The samples were analysed for methane content, pH value, redox potential, and concentrations of glucose, short-chain volatile fatty acids (sVFAs), ammonia, nucleobases, and protein. Furthermore, protozoa were counted and their motility evaluated.

Results: Preliminary comparison of treated and control incubators showed only minimal differences or none at all with respect to pH value, redox potential, overall production of microbial protein and methane or the total amount of sVFAs produced. As opposed to these results, the percentage deviations of the medians of the following parameters from untreated controls were noticeable (table 1).

Table 1: Fermentation performance after six hours of incubation (lower clinoptilolite level)

Parameter	Clinoptilolite supplementation as compared to control [%]
Acetate production [mmol/6 h]	minus 7.1[b] (n = 42)
n-Butyrate production [mmol/6 h]	plus 7.8* (n = 42)
i-Butyrate production [mmol/6 h]	minus 44.3[b] (n = 42)
i-Valerate consumption [mmol/6 h]	minus 12.2[b] (n = 42)
Total nucleobases production [mg/6 h]	minus 10.8[b] (n = 40)
Protozoal motility [score]	plus 7.1* (n = 26)

n - sample size; [b] not significant; * p <0,05 (Wilcoxon signed rank test)

Conclusion: Under the prevailing conditions the clinoptilolite addition does not seem to change the effectivity of rumen fermentation in general but rather causes a shift of the fermentation pattern probably as a reflection of a shift within the population of rumen microorganisms.

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72.

Effects of a monensin-releasing capsule and a blend of essential oils on white blood cell profile and function of periparturient dairy cows

Auswirkungen eines Monensin-freisetzenden Bolus und einer Mischung ätherischer Öle auf das Blutbild und die Zellfunktion der Leukozyten von Milchkühen in der peripartalen Phase

*Drong C., Meyer U., von Soosten D., Frahm J., Rehage J., Dänicke S. – Braunschweig/Hanover

Transition period displays the majority of health problems in dairy cows. Elevated concentrations of non-esterified fatty acids and ketone bodies seem to be aetiologically involved in an impaired function of neutrophils and leukocytes around calving (1). The ionophore antibiotic monensin was shown to improve the energy status and to reduce the ketosis incidence of high condition cows whereas the natural alternative essential oils failed to elicit any positive effect (2). The present work examines possible effects of supplements on immune cell populations and functions.

Methods: Sixty multiparous German Holstein cows were allocated six weeks ante partum (a.p.) to either high body condition score (BCS) 3.88 ± 0.1 (n=45) or low BCS 2.81 ± 0.1 (LC, n=15) group. High condition cows were overfed in the dry period with a 60% concentrate proportion in the daily ration in comparison to 20% in the LC group. After calving the concentrate proportion was raised from initially 30% to 50% in all cows. This increase was decelerated (3 vs. 2 weeks) in high condition cows to further stimulate post partum (p.p.) lipolysis. The high condition cows were subdivided into one control group (HC, n = 15), HC/MO group (n=15) receiving a monensin controlled-release capsule (Kexxtone, Elanco) and HC/EO group (n=15) receiving a blend of essential oils (CRINA Ruminants, DSM) from day 21 a.p. until day 56 p.p.. Blood samples were taken on day (d) -42, -14, -7, -3, 1, 7, 14, 21, 28, 35, 42, 56 relative to calving for white blood cell (WBC) profile analysis and flow cytometry. T-cell phenotyping was carried out by staining whole blood samples with monoclonal antibodies for CD4 and CD8. The capacity of polymorphonuclear leukocytes (PMN) to elicit oxidative burst is displayed in the $R123^+$ population indicating the portion of PMN that converted dihydrorhodamine 123 to the fluorescent metabolite rhodamine 123 (R123) via producing reactive oxygen species. Cell metabolic activity and the mitogen-stimulated proliferation of peripheral blood mononuclear cells (PBMC) were investigated on d -42, -7, 1, 7, 14, 21 and 56 by Alamar Blue assay. Parameters were evaluated in 3 periods (d -42 until calving, 1 until 14 days in milk (DIM), 15 until 56 DIM). Statistical analyses were performed by PROC MIXED procedure of SAS for repeated measures including experimental treatment, period and the interaction between these factors. $P < 0.05$ was assumed to indicate significant differences.

Results: There were no statistical differences between parameters of LC and HC group. HC/MO group displayed the lowest WBC count which was 19% lower than in HC and HC/EO group, respectively, over the whole trial ($p < 0.05$). A treatment x period interaction was found for $CD4^+$ subpopulations ($p < 0.05$) and the $CD4^+/CD8^+$ ratio ($p < 0.05$). Treatment did not affect the other parameters from cell culture and flow cytometry. WBC count was increased in period 2 ($p < 0.05$) driven by higher absolute numbers of granulocytes and lymphocytes. Basal viability of PBMC was higher in the second than in the third period ($p < 0.05$) while the proliferation after mitogen-stimulation increased from period 1 to period 2 ($p < 0.05$) and 3 ($p < 0.001$). Basal and stimulated $R123^+$ population dropped in period 2 and increased thereafter again ($p < 0.05$).

Conclusions: The improved energy status after monensin supplementation (2) was not reflected in notable changes of immunological parameters of the cows except for a lower number of WBC which might indicate a reduced activation of immune system. Changes in investigated parameters were rather related to the event of calving likely due to physical, endocrine and metabolic adaptations. Essential oils provided no benefit on mentioned parameters under present conditions.

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73.

Effects of peripartal energy supply and nicotinic acid supplementation on functional activity and gene expression of blood leukocytes and on anti-oxidative enzyme activity in serum of periparturient dairy cows

Effekte der peripartalen Energieversorgung und einer Nikotinsäureergänzung auf die funktionelle Aktivität und Genexpression von Blutleukozyten und auf antioxidative Enzymaktivität im Serum von peripartalen Milchkühen

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The periparturient dairy cow is challenged with severe metabolic and immunological changes during the transition from late gestation into early lactation. This transition is accompanied by an immunosuppression that renders the animal more susceptible to infections and metabolic disorders. Non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB), which peak shortly after parturition due to lipolysis, are known to impair immune cell functions. The well-known anti-lipolytic activity of niacin may have the ability to ameliorate this situation. In addition, niacin shows also anti-inflammatory and anti-oxidative effects that may be beneficial to the immune status of the cow. The study was conducted to examine the influence of peripartal differing energy levels and nicotinic acid supplementation on the functional activity of blood immune cells and their effect on the expression of oxidative stress-related genes in these cells, as well as on anti-oxidative enzyme activities in the serum.

Methods: Four feeding groups were established with a total of 47 German Holstein cows (29 multi-parous and 18 primiparous). They were fed either with a ration with a high concentrate proportion of 60% (HC) or a low concentrate proportion of 30% (LC). After parturition both concentrate levels were reduced to 30% and increased again to 50% either within 16 days (LC-group) or within 24 days (HC-group) to trigger cow groups differing in postpartum predisposition for developing lipolysis related metabolic disorders. Half of the animals received either 24 grams per day of nicotinic acid (LC-NA, HC-NA) or none for the control groups (LC-CON, HC-CON). Supplementation was applied from 42 days prepartum until 24 days postpartum. The trial period started 42 days before expected parturition and ended at 100 days in milk. Oxidative burst activity of polymorphonuclear leukocytes (PMN) and phagocytic activity of PMN and peripheral blood mononuclear cells (PBMC) were examined with flow cytometry. Oxidative stress related genes glutathione peroxidase 1 (GPX1), superoxide dismutase 2 (SOD2), and xanthine dehydrogenase (XDH) were analyzed with quantitative real time PCR on total RNA from blood leukocytes. Additionally GPX and SOD activities were examined photometrically in serum. Statistical evaluation was done using the MIXED procedure of SAS.

Results: For all measured variables a time dependency was observed which was mainly related to parturition ($p<0.030$). Parity influenced all measured variables except of PMN phagocytosis. Gene expression levels for GPX1, SOD2 and XDH were higher in cows than in heifers ($p<0.020$). Oxidative burst stimulation was higher in heifers than in cows ($p=0.036$). Serum activity of GPX was higher in cows ($p=0.012$) and serum activity of SOD was higher in heifers ($p<0.000$). The percentage of phagocytizing PBMC was higher in cows ($p=0.041$). A concentrate effect was found for GPX serum activity ($p=0.029$) meaning the HC group showed increased GPX activity compared to the LC group. Nicotinic acid supplementation tended to increase PMN and PBMC phagocytosis ($p<0.100$).

Conclusion: We could confirm that parturition is a period of multifold changes with considerable impact on immune cell functional activity and gene expression as well as on anti-oxidative enzyme activity in the serum. Parity in this context plays an important role, since animals differing in age had different prerequisites and functional abilities to respond to the stressful period of parturition. In terms of feeding effects we observed some concentrate level effects on anti-oxidative enzyme activities and a tendency for increased phagocytosis of blood leukocytes with nicotinic acid supplementation. This nicotinic acid effect however showed no interaction with the concentrate level.

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74.

Different dietary levels of NSP-degrading enzymes: effects on physico-chemical properties of digesta, excreta as well as on foot pad health of broilers

Effekte unterschiedlicher Gehalte an NSP-spaltenden Enzymen im Mischfutter für Masthühner auf die physikalisch-chemischen Eigenschaften von Chymus, Exkrementen sowie auf die Fußballengesundheit

Weiß H., *Kölln M., Neto R. M., Kamphues J. – Hanover/Antony

Introduction: NSP (=non-starch polysaccharides)-degrading enzymes are often used in poultry diets, not at least because of the reported positive effects on excreta/litter quality. This might be interesting regarding foot pad dermatitis (FPD), a frequent disease, mainly caused by wet litter1. The hypothesis of this study was that there might be a link between dietary level of NSP-degrading enzymes and excreta/litter quality as well as on foot pad health in broilers. Besides, effects of dietary enzymes in the digesta (viscosity) on changes in drying properties of excreta (water release, evaporation) should be tested.

Methods: 240 broilers (Ross 708) were divided into 4 treatments at the age of 9 days (each treatment with 3 replicates, so the birds were housed in 12 barns à 20 animals, littered with 1 kg wood shavings/m2). The pelleted diets were based on wheat (64%) and soybean meal (30%) and only differed in the use of NSP-degrading enzymes: Treatment 1 (control) was offered a diet without any NSP-degrading enzymes, whereas treatment 2, 3 and 4 received diets with 50, 100 and 500 % (of recommended dosage) of a conventional NSP-degrading enzymes containing product (RovabioTM Excel AP; added before pelleting). All animals received feed and water ad libitum and the intake was measured daily. Weekly, the body mass and footpad score (Score acc. to Mayne et al. 2007, modified) were recorded individually; the DM contents of excreta/litter were analysed in pooled samples of each replicate. Besides, there was an experimental in-vitro method tested to characterize the drying properties of excreta (water losses/evaporation of 10g excreta samples during 4h of "incubation" at 50°C). After slaughtering on day 37 digesta viscosity in the prox. small intestine (SI) was determined (method according to 4). Statistical analyses were performed by using the SAS® software (Cary, NC, USA), using the Ryan-Einot-Gabriel-Welsch-Test for normally distributed data and the Kruskal-Wallis-Test for not-normally distributed data. Pair-by-pair-comparisons were done with the Wilcoxon-Test.

Results:

Treatment (% of recommended enzyme dosage)	1 (0)	2 (50)	3 (100)	4 (500)
Arabinoxylan[1)] (g/kg diet as fed)	45.1 (total), 7.38 (soluble)			
Enzyme activity in the diet (U/kg)[2)]	0	551	1036	7595
Body weight (d 36, g)	$2252^{a} \pm 223$	$2212^{ab} \pm 233$	$2195^{b} \pm 367$	$2141^{b} \pm 255$
Total FCR (day 9-35)	$1.54^{a} \pm 0.011$	$1.52^{a} \pm 0.021$	$1.49^{a} \pm 0.032$	$1.53^{a} \pm 0.034$
DM content of excreta (d 35, %)	$16.1^{a} \pm 2.65$	$15.9^{a} \pm 1.70$	$16.5^{a} \pm 1.51$	$16.9^{a} \pm 0.624$
DM content final litter (d 37, %)	$38.5^{a} \pm 1.14$	$39.6^{a} \pm 4.05$	$37.5^{a} \pm 0.95$	$36.8^{a} \pm 3.15$
Digesta viscosity (cP, prox. SI)	$5.10^{a} \pm 0.959$	$5.05^{ab} \pm 0.673$	$4.24^{b} \pm 0.794$	$3.42^{c} \pm 0.811$
FPD-Score[3)] (d 36)	$3.93^{ac} \pm 0.618$	$3.66^{b} \pm 0.692$	$3.82^{ab} \pm 0.722$	$4.08^{c} \pm 0.643$

[a,b,c] indicate significant differences in one line ($p<0.05$); [1)]determined by Kluge (2015), analysed in diet 1; [2)]Adisseo, France; [3)]low values → less "pathological" changes (0=healthy; 7=over half of foot pad necrotic)

The analysed dietary enzyme activities showed the intended differences. Viscosity of digesta in the prox. SI was decreased significantly by using higher dietary enzyme dosages. Birds of treatment 1 had the highest mean BW at day 37, but there were no significant differences regarding FCR and DM content of excreta and litter. The results regarding drying properties of excreta showed no clear effects of dietary treatment. Animals of treatment 4 had sign. higher (=unfavourable) foot pad scores compared to treatment 2 and 3.

Conclusion: In this experiment the dietary use of NSP-degrading enzymes at the recommended level caused the numerically most favourable FCR. Using 500% of the recommended dosage seemed to have disadvantageous effects on foot pad health. As water binding capacity and release of water (evaporation) of litter is predominantly determined by excreta (and not by litter), the undigestible parts of the diet, but also the surplus of nutrients excreted are factors that are worth mentioning when DM content of litter is on debate. The question is still not answered whether degraded NSP in the excreta contribute to its water binding capacity; improved in-vitro tests are planned to study the dietary impact on evaporation (determining predominantly "litter quality").

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75.

Influences of different dietary levels of Mannan-degrading enzymes on performance, occurrence of *C. perfringens* in small intestine contents, as well as on excreta/litter quality and foot pad health in broilers

Einfluss unterschiedlicher Gehalte von Mannanase im Mischfutter auf die Leistung, das Vorkommen von C. perfringens im Dünndarmchymus sowie die Exkremente-/Einstreuqualität und die Fußballengesundheit von Broilern

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Introduction: Mannans are special non-starch polysaccharides in soybean meal, the common protein source in broiler diets. These non-starch polysaccharides may decrease the utilisation of nutrients and subsequently increase litter moisture (1). By including Mannan-degrading enzymes in the diets these negative effects could be minimized. The litter moisture is known as the main factor to induce foot pad dermatitis (2). The hypothesis of this study is that the excreta and litter quality can be improved by the use of Mannanase in broilers' diets. Subsequently an improved foot pad health was expected.

Material and Methods: 264 one day old broiler poults (Ross 308, both sexes) were divided into four treatments (in each three replications with two groups à 17 broilers and one group à 32 animals, that means 66 per treatment). All groups received feed and water ad libitum and the intake was measured daily. The feed was based on wheat (64 %), soybean meal (30 %) and soybean oil (2 %). Minerals and vitamins were included in a premix (4 % of the diet). The nutrient contents of the different diets were similar to each other and covered the requirements. The control group (I) got the diet without Xylanase and Mannanase, diet of treatment II contained only Xylanase, while the other treatments (III and IV) received diets with increasing dosages of Mannanase (100/200 % of recommended dose) and common levels of Xylanase. The used Mannan-degrading enzyme was an Endo-1,4-β-D-Mannanase. Every week each animal was weighted and a FPD-Score (3) was compiled. Furthermore pooled excreta and litter samples of all groups were taken weekly and analysed on the DM-content, as well as the pH. At necropsy (day 22/37 of life, in total 32 birds per group) small intestine content was collected and cultivated for *C. perfringens* on NPC-agar. Statistical analysis was done by using the SAS® software (Cary, NC, USA) (PROC GLM / PROC NPAR1WAY / PROC FREQ).

Results and Discussion:

Table 1: Results regarding enzyme activity in the diet, performance parameters, excreta/litter quality and FPD-Score in broilers

Treatment/group	I	II[1)]	III[2)]	IV[3)]
Body weight, d 36 (g)[4)]	$2528^{ab} \pm 291$	$2539^{ab} \pm 543$	$2500^{a} \pm 335$	$2642^{b} \pm 298$
DM content of excreta, d 33 (g/kg)	162 ± 8.86	161 ± 4.45	158 ± 13.1	158 ± 6.81
DM content "final litter", d 38 (g/kg)	402 ± 5.01	391 ± 6.69	425 ± 32.9	399 ± 19.2
FPD-Score, d 36[5)]	$3.35^{a} \pm 1.18$	$3.14^{a} \pm 1.43$	$2.26^{b} \pm 1.56$	$3.24^{a} \pm 1.56$
C. perfringens positive samples, (%)[6)]	6.45^{a}	51.6^{b}	25.8^{ab}	12.9^{a}
C. perfringens, (cfu/g)[7)]	$163^{ab} \pm 138$	$183^{ab} \pm 158$	$174^{ac} \pm 210$	$621^{bd} \pm 841$

[1)]one replication was not included in the results regarding FPD-Score and DM-content of final litter; [2)]Enzyme activity of Mannanase 0.087 and [3)]0.176 (MU/kg), determined by IFF Braunschweig, Germany; [4)]$n_{group\ I/II/III/IV}$=51/51/49/51; [5)]$n_{group\ I/II/III/IV}$=51/38/49/51; [6)]n=31/group; [7)]positive samples only; [a, b, c, d]indicate significant differences (p

The doubling of Mannanase resulted in significantly higher final body weight. Inspite of only slightly improved DM content in the final litter, in group III the most favourable FPD-Scores were observed in that group. The most interesting results occurred regarding *C. perfringens* (group I fed a diet without any enzyme had the lowest percentage of positive animals).

Conclusion: The findings in group III regarding the DM content of final litter and the foot pad health of broilers seem to be worth to continue with investigations. Further studies need to be done to prove the mechanisms behind the observed effects.

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76.

Relative efficacy of bentonite-montmorrilonite and dried yeast cells as biosystemic sequestering agents at high dietary aflatoxin load in turkey poults

Wirksamkeit von Bentonit-Montmorillonit und getrockneten Hefezellen als Komplexbildner bei hohen Gehalten an Aflatoxin im Futter für Putenküken

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Aflatoxin (AFL) is sporadically experienced in poultry diets, resulting in depressed performance. Thus additive binders or active biomolecules suppressive to AFL noxious effects are routinely supplemented to practical feeds. Nevertheless there is little comparative data on the relative efficacies of available supplements. Therefore we compared the efficacy of proprietary Bentonite-Montmorillonite (Bioad®) and dried yeast cells (Promotop®) used in the industry to prevent or alleviate aflatoxicosis. Turkey poults were used for this investigation.

Methods: One hundred and ninety two 14 d poults were distributed in a completely randomized design to 6 diets, 4 replicates and 8 poults per experimental unit. Treatments were: diet 1 (positive control, as uncontaminated standard diet, PC), diet 2 (negative control, NC), made by partial replacement of uncontaminated corn in the PC with corn cultured with toxigenic Aspergillus flavus to achieve 150 ppb total AFL), diet 3 (NC + 1.5 g/kg Bioad®), diet 4 (NC+ 3 g/kg Bioad®), diet 5 (NC + 1.5 g/kg Promotop®), diet 6 (NC + 3 g/kg Promotop®). Aflatoxin level was confirmed in individual diets by gas liquid chromatographic analysis. Data were subjected to analysis of variance and means were separated based on Duncan multiple range test (5% α-value). All animal procedures were approved by the animal welfare regulations unit and conducted in accordance to the guide for the care and use of agricultural animals in research and teaching (FASS, 2010).

Results: At d 44, high dietary ingestion of aflatoxin was detrimental to feed intake, BW gain and fcr ($P < 0.05$). These parameters however were significantly improved relative to NC by both supplements at each level evaluated ($P < 0.05$), although their benefits could not match the PC in terms of BW gain ($P < 0.05$). Aflatoxicosis resulted in 56% mortality in the unsupplemented diet which was attenuated to 0 in both diets supplemented with Bioad®, while the use of Promotop® reduced mortality to 12% and 0 in diets 5 and 6, respectively. On the other hand blood haematology and serum biochemistry assayed were not sensitive to aflatoxin intoxication. We interpreted the efficacy of each agent as the slope of a second order polynomial fitted to the BW gain (g/30 d) and daily intake of each agent in mg/30 d (Figure 1, left). F test (5% α-value) showed that the supplements had similar efficacy at the levels tested ($P > 0.05$). However the marginal efficiency computed as the first derivative of the polynomial fit suggested different utilization efficiencies (Figure 1, right).

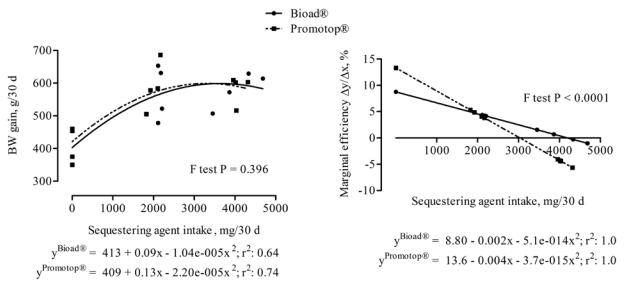

Figure 1 Effects of proprietary bentonite -montmorrilonite (Bioad®) or dried yeast cells (Promotop®) as sequestering agents on body weight gain (left) and marginal efficiency of body weight gain (right) in turkey poults challenged with 150 ppb of dietary aflatoxin

Conclusion: This study demonstrated that 150 ppb AFL is toxic in poults and could be ameliorated by dietary supplementation of the tested sequestering agents but at varying efficiencies which decreases with increasing rate of intake.

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77.

Zootechnical and gastrointestinal effects of brown algae *Laminaria saccharina* in weaned piglets

Zootechnische und gastrointestinale Effekte der Braunalge Laminaria saccharina beim Absetzferkel

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Introduction: *Laminaria saccharina* is a marine brown algae grown mainly in East Asia for production of alginate for the food industry. Feed value of these algae is estimated to be low due to the high content of non-starch-polysaccharides. Nevertheless, algae are attributed beneficial health effects on the gastrointestinal tract due to their potential prebiotic actions. Up to date, systematic investigations on the presumed gastrointestinal effects are still lacking.

Methods: Three 5week studies with 48 weanling piglets each were conducted. Study 1 was designed to check the general acceptance of algae material in feed. In study 2, dose-response effects of increasing algae material in the diet were tested. Finally, study 3 should deeply uncover the intestinal effects and mode of action of supplemented algae. Animals were fed common basal feed mixtures supplemented with dried algae powder (AP) from *Laminaria saccharina* and/or diatomaceous earth (DE) as follows: study 1: 2.5% DE (control), 2.5% sun dried AP, or 2.5% drum dried AP; study 2: Rising dietary levels of drum dried AP at the expense of DE: (0%, 1.7%, 3.3%, 5%); study 3: 5% drum dried AP (A1), 5% drum dried AP treated by β-radiation in order to reduce carbohydrate molecule size (A2), 5% DE (negative control; NC), or 100% basal feed (positive control; PC). Body weight and feed intake as well as fecal score were recorded (study 1 and 2). Furthermore, total tract digestibility of nutrients using TiO_2 was evaluated (study 2 and 3). In study 3, animals were sacrificed to investigate intestinal microbiology, histology, and mRNA expression of related genes. Statistics were based on a 2way-ANOVA (feeding group and block (litter mates of same sex)).

Results: Compared to control piglets fed DE, algae did not affect weight gain in neither study but tended to reduce feed intake resulting in an improved feed conversion (5% in study 1 ($p<0.01$), up to 7% in study 2 ($p<0.01$), 6% in study 3 ($p<0.02$)). In study 3, PC and animals fed algae performed equally. Fecal score was not affected at any time in neither study.

Digestibility of nutrients and hence dietary energy were quite low in native algae, but were considerably improved through β-radiation (3.6 vs. 9.5 MJ ME/kg DM, $p<0.01$ (study 3)).

In study 3, algae reduced length of small intestine by 9% compared to PC ($p<0.05$), and villi length as well as crypt depth in jejunum. Dry matter content of chyme in terminal ileum was considerably lower (12% vs. 16%, $p<0.05$). Within dry matter of ileal chyme the counts of bifidobacteria, lactobacilli, enterococci, C. perfingens, E. coli and enterobactgeriaceae were reduced but their ratio remained unchanged. pH, ammonia, lactate as well as concentration and pattern of volatile fatty acids and biogenic amines did not reveal systematic changes due to feeding algae. Significant ($p<0.05$) changes in mRNA expression profiles due to algae addition were observed in mesenterial lymph nodes (IL-5 (up)), jejunum ((CDK4 (up), Occludin (down), Ubiquitin (down)) and ileum (Occludin (up), Histone H3 (down)).

Conclusion: Despite low feed value, algae addition resulted in remarkably good zootechnical performance equaling control animals fed a highly digestible basal feed. These results suggest a particulate benefit to the gastrointestinal health due to algae supplementation. The possibly unfavourable reduction of intestinal surface induced no negative effects (e.g. lower absorption of nutrients) as animals performed similar to control animals. However, divergent results on gene expression level as well as patterns of intestinal microbes and their metabolites suggest a growth promoting effect, but not a prebiotic mode of action.

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Influence of DON (Deoxynivalenol) on the piglets immune system with due regard to sodium sulfite decontaminated feed. *Ex vivo* results in the LPS challenge

Einfluss von Deoxynivalenol (DON) auf das Immunsystem des Ferkels unter besonderer Berücksichtigung einer Na2SO3-Dekontaminierung des Futters. Ex vivo Ergebnisse in der LPS Challenge

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Pigs respond very sensitive to the presence of the mycotoxin deoxynivalenol (DON) in the diet, especially with a reduction in feed intake and with an immuno-modulation. A recent study reported that sodium sulfite (Na_2SO_3, SoS) treatment of DON contaminated feed reduced the toxicity of DON as proven by an improved feed intake (1). In the present study, the effects of feeding a DON contaminated diet, either untreated or SoS treated on porcine peripheral blood mononuclear cells (PBMCs) were examined in lipopolysaccharide (LPS) stimulated and unstimulated piglets.

Methods: 80 piglets (5-7 kg BW) were equally divided into four experimental groups and fed with different diets (Fig. 1): CON- (control diet), CON+ (diet containing SoS treated maize), DON- (diet containing untreated contaminated maize; 6 mg DON/kg), and DON+ (diet containing SoS treated contaminated maize; 0.8 mg DON/kg). After 37 days, half of each group (n=10) was injected intraperitoneally either with 0.9% NaCl/kg BW or with 7.5 µg LPS/kg BW (volume ~ 6.5 mL). Two hours after injection, the blood samples were collected and PBMCs were isolated on a Ficoll gradient. PBMC were cultivated at 37°C and 5% CO_2 and treated for 72 h as follow: Control (medium RPMI 1640), ConA (12.5 µg/ml concanavalin A), PHA (10 µg/ml phytohaemagglutinin), and *E.coli* (O111:B4) LPS (1 µg/ml LPS). Thereafter supernatants were collected and the proliferative capacity and the nitric oxide (NO) release level were evaluated using Alamar Blue and Griess assays. Data were statistically analysed with a 3-factorial ANOVA (feed, SoS, LPS) and differences (Student's *t*-test) were considered significant at $p \leq 0.05$ and as a trend at $0.05 < p < 0.10$.

Results: *Ex vivo*, ConA and PHA stimulation strongly increased PBMC proliferation (Fig. 2A), whereas LPS stimulation *ex vivo* hardly elicited any proliferative effect compared to control. SoS treatment in non-challenged pigs *in vivo* significantly decreased PBMC proliferation for ConA and PHA stimulation (DON- vs. DON+: $p<0.05$), whereas this was only a tendency for control and LPS-treatment *ex vivo*. However, this proliferative depression in ConA and PHA-treated cells *ex vivo* was absent in the PBMCs of their LPS-challenged counterparts. The NO release level in PBMC *ex vivo* was neither affected by diet, SoS treatment or LPS challenge (Fig. 2B).

Conclusions: The results indicate that *in vivo* DON exposure had no impact to *ex vivo* PBMCs proliferation. Besides, SoS treatment *in vivo* in non-challenged pigs depressed the PBMCs proliferative capacity. However, this effect was annulled in LPS stimulated pigs in DON+ group under ConA and PHA stimulated conditions. Further analyses elucidating the observed effects are in progress.

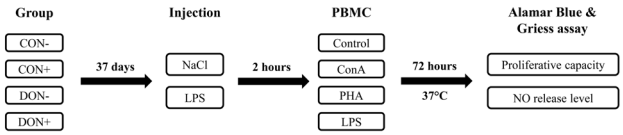

Figure 1: Schematic of the experimental process

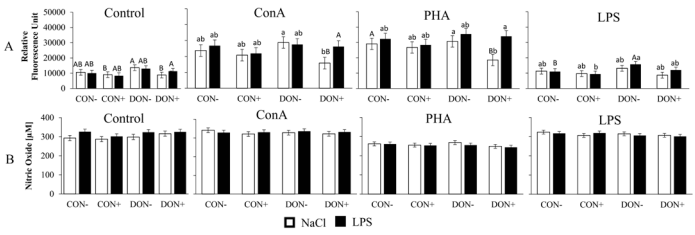

Figure 2: Experimental results obtained from Alamar Blue (panel A) and Griess assays (panel B). Differences (Lsmeans + SEM) were considered significant at $p \leq 0.05$ (letters), a trend was noted when $0.05 < p < 0.1$ (capital letters).

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79.

Influence of dietary Quebracho tannin extract on the fatty acid composition of milk fat in dairy cows

Einfluss von Quebrachotanninextrakt in der Ration auf die Fettsäurezusammensetzung des Milchfettes von Kühen

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Beside the ability of condensed tannins to form reversible complexes with feed proteins, they are also able to inhibit rumen microbial growth and activity. Hence, milk fatty acid (FA) composition could be altered caused by modification of ruminal biohydrogenation (RBH) of unsaturated FA (USFA). Therefore, the aim of our study was to evaluate the effect of Quebracho tannin extract (QTE) on the content of saturated (SFA) and USFA as well as on linoleic acid, linolenic acid, rumenic acid, vaccenic acid, and stearic acid in milk fat as indicators for modification of RBH.

Methods: Fifty Holstein dairy cows were divided into two groups according to milk yield, lactation number, days in milk, and body weight (33.2 ± 8.2 kg/d, 2.30 ± 1.59, 114 ± 73 d, and 638 ± 58 kg, respectively). Cows were fed a basal diet as total mixed ration, based on grass and maize silage and concentrates (65:35). To create three experimental treatments, the basal diet (CON) was supplemented with QTE at levels of 15 (QTE_{15}) and 30 g/kg DM (QTE_{30}).The study was performed with six experimental periods (P) of 21 d (13 d of adaptation and 8 d of sampling). From d 14 to 21 of each P, milk samples were collected from every cow once a day, alternating between morning and afternoon milking, and pooled per cow according to milk yield. Milk FA composition (g/100 g FA) of pooled samples was determined by gas chromatography. Due to technical limitations only two treatments could be tested simultaneously. Therefore, treatments were arranged along the six P as follows: P1, CON-CON; P2, QTE_{15}-CON; P3, CON-QTE_{15}; P4, QTE_{30}-CON; P5, CON-QTE_{30}; P6, CON-CON, respectively. All statistical analysis were run for 49 cows using the statistical program R. In order to adjust the treatment effect, milk yield (kg/d) and lactation number included as covariates and analysis of covariance was conducted. Multiple contrast tests were run to test for interactions between P and treatment and to determine treatment effects. Finally, QTE effect on milk FA contents was estimated with an average milk yield and lactation number of 32.4 kg/d and 2.3, respectively, based on linear regression equation. Significant effects were declared at $P<0.05$.

Results: Milk SFA content decreased and, simultaneously, milk PUFA content increased when feeding 15 and 30 g QTE/kg DM. The ratio SFA/USFA showed a decrease with increasing level of QTE. Both QTE treatments resulted in a stepwise increase of linoleic (*c9c12*-C18:2) and linolenic acid content (*c9c12c15*-C18:3) and decrease of rumenic acid content (*c9t11*-C18:2, conjugated linoleic acid). Compared with CON, both QTE treatments decreased the content of vaccenic acid (*t11*-C18:1). The end product of RBH, stearic acid (C18:0), was higher in milk fat when feeding 15 and 30 g QTE/kg DM.

FA	Intercept			Slope of covariate		Estimated FA content		
(g/100 g FA)	CON[1]	QTE_{15}[2]	QTE_{30}[3]	b_1	b_2	CON	QTE_{15}	QTE_{30}
SFA	63.3^{a}±0.19	62.7^{b}±1.39	61.7^{c}±1.35	1.201*	0.020*	66.7	66.1	65.1
PUFA	4.17^{c}±0.18	4.37^{b}±0.18	4.83^{a}±0.17	-0.142*	0.013*	4.26	4.46	4.92
SFA/USFA	1.78^{a}±0.12	1.74^{b}±0.12	1.64^{c}±0.12	0.113*	0.002*	2.10	2.06	1.96
Linoleic acid	1.79^{c}±0.08	1.98^{b}±0.08	2.31^{a}±0.08	-0.058*	0.004*	1.78	1.97	2.30
α-Linolenic acid	0.63^{c}±0.03	0.67^{b}±0.03	0.78^{a}±0.03	-0.022*	0.0001	0.58	0.62	0.73
Rumenic acid	0.50^{a}±0.05	0.46^{b}±0.05	0.42^{c}±0.05	-0.032*	0.003*	0.52	0.48	0.44
Vaccenic acid[#]	1.32^{a}±0.21	1.20^{b}±0.21	1.20^{b}±0.20	-0.088*	0.014*	1.56	1.44	1.44
Stearic acid	10.9^{c}±0.55	11.2^{b}±0.55	12.3^{a}±0.54	0.002	-0.029*	9.97	10.3	11.4

[1] n=196; [2] n=49; [3] n=49; b_1, slope of covariate lactation number; b_2, slope of covariate milk yield (kg/d); PUFA, poly unsaturated fatty acids; [#]Detected incl. *t10*-C18:1; [abc]Different superscripts indicate significant treatment effects ($P<0.05$), *indicates significant effect of covariates ($P<0.05$)

Conclusions: Dietary QTE have the potential to modify RBH. The increase of linoleic and linolenic acid and the decrease of rumenic and vaccenic acid in milk fat may indicate for modification at the initial step of isomerization. However, it is questionable whether the effects of QTE on milk fat composition (e.g. increasing milk PUFA content) have the potential to improve the milk fat quality.

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80.

Combined effect of essential oils and biotin on body condition and serum markers of energy metabolism in transition dairy cows

Kombinierter Effekt von ätherischen Ölen und Biotin auf die Körperkondition und Serummarker des Energiestoffwechsels bei Milchkühen in der Transitphase

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Several management and feeding factors are currently applied to decrease the incidence of ketosis in postparturient cattle. A leading role has the provision of extra gluconeogenic precursors either as feed additives or by modulating ruminal fermentation to increase propionate production. Supplementation with essential oils has been reported to increase ruminal propionate production in some studies, which was verified for beef cattle in a recent meta-analysis (1). Biotin is an essential cofactor of propionyl-CoA and pyruvate carboxylases. Biotin could thus promote gluconeogenic activity and add to the beneficial action of essential oils. Therefore, the present study was conducted to test whether the combined dietary supplementation with essential oils and biotin in the transition phase could improve postpartum energy metabolism.

Methods: Thirty five multiparous Holstein-Friesian dairy cows on a commercial dairy farm received a mixed ration based on grass and corn silage plus 1 kg of concentrate during the close-up period and up to 7 kg of concentrate at peak lactation. Cows were allocated into one control group (n = 16) and one CrinaB group (n = 19). Cows in the CrinaB group received 1 g/d CRINA® Ruminants (DSM, Kaiseraugst, SUI) and 40 mg/d ROVIMIX® Biotin (DSM) mixed in 1 kg of concentrate from day D-21 ± X (21 days before expected calving) until D37 after calving; whereas, cows in the control group received equivalent amounts of concentrate without such supplements. Blood samples were taken once weekly from 3 wk before the expected calving up to 8 wk after the parturition. Body condition score (BCS) and backfat thickness (BFT) were recorded each time when a blood sample was taken. Data were compared at each time point using *t*-test and are presented as means ± SEM.

Results: Cows in the CrinaB group maintained a better BCS after calving, which was significant at D30 (Control: 2.61 ± 0.09; CrinaB: 2.86 ± 0.10; $P < 0.05$) and seen as a trend on D16, D23, D37, D44, D51 and D58 ($P < 0.1$). The improved BCS was associated with a numerically higher BFT towards the end of the study period (D51, Control: 3.34 ± 0.12 cm; CrinaB: 3.59 ± 0.10 cm; $P = 0.11$). Serum concentrations of glucose, non-esterified fatty acids, β-hydroxybutyric acid, triglycerides, cholesterol and urea did not differ between groups. Cows of the CrinaB group showed a faster decrease of serum bilirubin after parturition, which was significant on D30 (Control: 2.40 ± 0.36 µmol/L; CrinaB: 1.62 ± 0.17 µmol/L; $P < 0.05$).

Conclusion: The obtained data give evidence that supplementation with the tested essential oil preparation and biotin can be beneficial to prevent excessive mobilization of body tissue in early lactation. The effect was predominantly visible on BCS but not significant for BFT, which may suggest that primarily decrease of lean body mass was reduced. A positive effect of CrinaB on postpartum bilirubin concentrations might indicate positive effects on the detoxifying capacity of the liver.

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81.

Supplementation of dairy cows with conjugated linoleic acid: effects on the mRNA abundance of genes related to protein synthesis and proteolysis in the skeletal muscle

Supplementierung von Milchkühen mit konjugierten Linolsäuren: Effekte auf die mRNA-Menge von mit der Proteinsynthese und Proteolyse assozierten Genen in der Skelettmuskulatur

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Skeletal muscle, the main labile source of amino acids in the body, plays a major role in homeostasis. The balance between skeletal muscle protein synthesis and proteolysis is controlled via several pathways whereby the mammalian target of rapamycin (mTOR) and the ubiquitin-proteasome system (UPS) are considered as the major regulators of protein synthesis and protein degradation, respectively (1). A preservative effect of dietary conjugated linoleic acids (CLA) on age-associated skeletal muscle loss was reported in mice (2). Thus, the aim of this study was to characterize the expression of key components of mTOR signaling and UPS-related genes in skeletal muscle of dairy cows during late gestation and subsequent lactation, and to test the potential effects of a dietary supplement with CLA.

Methods: Thirty German Holstein cows receiving 100 g/d CLA (5 primiparous and 11 pluriparous cows; Lutrell pure, BASF, Germany; each 12% of *trans*-10, *cis*-12 and *cis*-9, *trans*-11; CLA) or a control fat supplement (4 primiparous and 10 pluriparous cows; Silafat, BASF; CTR) from days in milk 1 to 182 were studied. Muscle biopsies were collected from *M. semitendinosus* on day (d) -21, 1, 21, 70, 105, 182, 196, 224, and 252 relatives to calving. The mRNA was quantified by real-time PCR for the key components of mTOR signaling pathway: mTOR, eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), ribosomal protein S6 kinase (S6K1); and of the UPS: atrogin-1 and muscle ring-finger protein 1 (MuRF-1), known as two major muscle-specific E3 ubiquitin ligases. Data were analyzed by the MIXED procedure of SAS with treatment, time, parity, and interaction of treatment × time and treatment × parity as fixed effects and cow as random effect. The threshold of significance was set at $P < 0.05$.

Results: The muscle mRNA abundance of key components of mTOR and UPS-related genes were neither affected by the CLA supplement nor by parity. The mRNA abundance of mTOR in the CTR group increased from d -21 to d 1 ($P = 0.02$), followed by a decline to nearly prepartum values by d 182, and increased thereafter. Expression of mTOR mRNA increased 1.88-fold in the CLA group from d 1 to d 182, and then declined about 3-fold by d 252. There was a significant interaction ($P < .0001$) of CLA supplementation × time due to the increase of mTOR mRNA values on d 70, 105, and 182 and the decrease after end of supplementation on d 224 and 252. The mRNA abundance of 4E-BP1 changed over time ($P < .0001$) and followed a similar pattern in both groups, i.e. they showed an increase from d -21 to d 1, then a decrease until d 21 and unchanged values thereafter. Time-related changes in the mRNA abundance of S6K1 were noted in both experimental groups ($P < .0001$). There were interactions between treatment × time and time × parity for the mRNA abundance of S6K1 ($P = 0.004$ and 0.002, respectively). In CTR cows, a 2.20- and 3.61-fold increase in atrogin-1 and MuRF1 mRNA were observed from d -21 to d 1, followed by a decline by d 21 and then remained largely unchanged. The mRNA abundance of atrogin-1 and MuRF-1 followed a similar trend in the CLA group; a 1.88- (atrogin-1) and 2.35-fold (MuRF-1) increase were noted from d -21 to d 1, then a decrease until d 70, and another increase by d 224. There was an interaction ($P < 0.001$) of treatment × time, because CLA cows had more MuRF-1 mRNA than CTR cows on d 224.

Conclusion: The increased mRNA abundance of atrogin-1 and MuRF-1 around calving may reflect upregulation of the UPS system at the level of the mRNA, and thus proteolysis might have been stimulated to provide extra amino acids to support protein synthesis in mammary gland or other tissues. Upregulation of mTOR expression in the CLA group during the supplementation period might be linked to a higher protein synthesis in the muscle tissue of these cows that warrants further investigations.

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82.

Cluster analysis of abundance of total bacteria in the rumen of bulls after fattening with varying structural value of diets

Clusteranalyse zur ruminalen Bakteriendichte von Bullen nach der Mast mit unterschiedlichem Strukturwert des Futters

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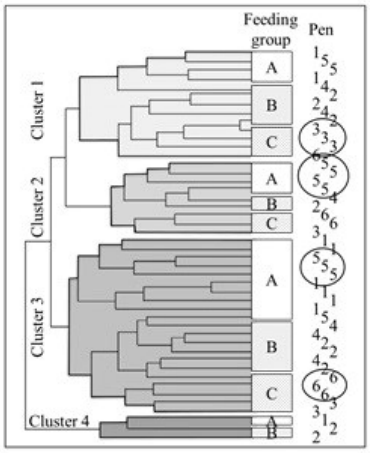
Introduction: The rumen microbiome and its interaction with nutrition is not well understood. The same applies to the relevance of genetic background and environmental factors occurring during life history and possibly imprinting the microbiome. In this context, a cluster analysis was done on bacterial abundance in the rumen of bulls for fattening measured in a large feeding study.

Method: 70 growing German Fleckvieh bulls (Start: 517 kg body weight) were included in a 191d fattening study. Animals were housed in 6 pens (n=11 to12) and were fed 3 diets differing in physically effective neutral detergent fiber: (A) pen 1+5: 294g/kg DM; (B) pen 2+4: 270g/kg DM; (C) pen 3+6: 246g/kg DM. Finally, animals were slaughtered (Slaughtering: 800 kg body weight). Rumen fluids as well as rumen mats were sampled, freeze-dried and analyzed for total bacteria via real-time quantitative PCR. Data sets from 67 animals were submitted to hierarchical cluster analysis using XLSTAT (Addinsoft, USA). A further cluster analysis was done with a subset of 9 pairs of animals which descended from the same farm, but received different dietary treatments and were housed in different pens.

Results: The whole data set (n=67) revealed 4 main clusters with high distance (82%). These clusters were not associated with dietary treatment or with time point of sampling or analytical processing. Within main clusters, sub-clusters grouped very precisely according to dietary treatments (indicated by A, B, C). Within these sub-clusters, individual data sets tended to cumulate with pen number (indicated by circles). The subset of data involving 9 pairs of animals revealed 4 clusters of each one pair showing very low distance (22%) between pair members (Data not shown). The remaining animals did not group systematically.

Conclusion: Dietary treatment clearly affected the rumen microbiome. But also other factors exerted a strong and possibly long-term impact on the microbiome. One of these factors might be common life history of animals within (e.g. within the same pen). Descent from the same farm did not determine the microbiome in half of analyzed cases, but in the other half of cases the impact was very strong. It might be speculated that this was due to genetic similarity and/or certain treatments in early life (nutrition, medication, etc.) imprinting the rumen microbiome.



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83.

Rumen protozoal numbers in dairy cows fed total mixed ration or grazing

Vergleich der Protozoenkonzentrationen im Pansen von Milchkühen bei TMR-Fütterung und Weidegang

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Protozoa can represent up to 50% of the total microbial biomass in the rumen (1) and have an essential impact on rumen fermentation. Abundance of protozoa depends mainly on feed composition. The objective of this study was to evaluate how transition from total mixed ration (TMR) feeding to grazing influences rumen protozoal numbers at different sampling times and sites in the rumen.

Methods: Ten rumen- and duodenum-fistulated dairy cows (German Holstein) were randomly assigned to either a confinement group (CG; n = 5) or a pasture group (PG; n = 5). The CG had *ad libitum*access to a TMR (35% maize silage, 35% grass silage and 30% concentrate; DM basis) during the entire experimental period of 10 weeks (w1-10). The PG was slowly adapted from TMR to continuous grazing on a ryegrass dominated pasture: w1: TMR, w2: TMR + 3 h/d on pasture, w3+4: TMR + 12 h/d on pasture, w5-10: 24 h/d on pasture + 1.75 kg DM concentrate/d. Protozoal numbers were determined in rumen fluid from the medial (upper 10 cm of solid phase) and ventral site (liquid phase) of the rumen. Samples were taken once a week after morning and afternoon milking. In w9, rumen fluid was additionally taken every 3 h over 24 h from the ventral rumen to investigate diurnal changes. Rumen fluid was diluted with methyl-green-formalin-salt solution and protozoa were counted in triplicate using a microscope and a counting chamber. Protozoa were grouped into holotricha and total protozoa (holotricha + entodiniomorphs). Statistical analysis was done using ANOVA and t-tests ($\alpha = 0.05$) with the MIXED procedure of SAS (version 9.4)

Results: In w3+4 total protozoal numbers were significantly higher or tended to be higher in CG than PG. Total protozoal number did not significantly differ in w1-2 and w5-10. In both groups total protozoal numbers were significantly or as a trend higher in the medial than in the ventral site of the rumen. In the course of a day the highest number of total protozoa in rumen fluid was detected at 8:00 in CG ($248 \cdot 10^3$/ml; 95% confidence interval (CI): 209-290) and at 9:30 in PG ($234 \cdot 10^3$/ml; CI: 198-272). Lowest total protozoal numbers in rumen fluid were detected at 2:00 in CG ($122 \cdot 10^3$/ml; CI: 96-152) and at 18:30 in PG ($160 \cdot 10^3$/ml; CI: 130-193).

Numbers of holotricha were significantly higher in PG than CG only at two sampling times in w1-4. In w6-10, significantly more holotricha were detected in PG than CG. The number of holotricha was significantly higher or tended to be higher in the ventral than medial rumen. In both groups highest numbers of holotricha in rumen fluid were detected in the morning: in CG at 8:00 ($3.8 \cdot 10^3$/ml; CI: 1.0-9.9) and in PG at 6:30 ($43.9 \cdot 10^3$/ml; CI: 24.4-72.2). Lowest numbers were detected during the night: at 2:00 in CG ($0.1 \cdot 10^3$/ml; CI: 0.0-0.9) and at 0:30 in PG ($0.7 \cdot 10^3$/ml; CI: 0.0-3.1).

Conclusions: Adaptation from TMR to grazing increased the number of holotrich protozoa but not total protozoa. Changes were most likely related to a higher intake of soluble carbohydrates contained in the fresh grass. Abundance of protozoa changed during a day and depended on rumen site. Therefore, sampling time and site in the rumen should be strictly standardised in studies investigating effects on rumen protozoal numbers..

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84.

Effect of glyphosate residues in animal feed on rumen pH-value, short chain fatty acids and rumination time of dairy cows

Einfluss von Glyphosatrückständen in Futtermitteln auf den Pansen pH-Wert, flüchtige Fettsäuren und die Wiederkauaktivität bei Milchkühen

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Glyphosate is currently one of the most controversially discussed herbicide. It inhibits the aromatic amino acid biosynthesis in plants and is suspected to influence the rumen microflora and consequently rumen fermentation. The aim of this study was to examine the effect of glyphosate residues in animal feed on rumen fermentation characteristics.

Methods: A total of ten cannulated German Holstein cows were divided into two different feeding groups with five cows each. The experiment lasted 16 weeks and was subdivided into three periods: period 1 (week 0, pre-treatment period), period 2 (week 5 to 8) and period 3 (week 14 to 15). In period 1 all cows received a total mixed ration (TMR) consisting of 30% maize silage, 30% grass silage, 40% concentrate (on a dry matter basis) for *ad libitum* consumption. During period 2 and 3 cows were divided into two groups fed *ad libitum*, either an uncontaminated control TMR (CON) or a glyphosate contaminated TMR (GLY). The TMRs were composed of 21% maize silage, 42% grass silage, 30% concentrate and 7% straw (on a dry matter basis). Straw and concentrate components originated from wheat and peas with or without glyphosate treatment were used. During the experimental periods pH-value in the ventral sac of the rumen was measured using a submersible continuous ruminal pH measurement system (1) (Dascor Inc., Escondido, CA, USA) and the rumination time (RT) was determined simultaneously in period 2 and 3 by a sensor-based automatic measurement system (Rumiwatch, Liestal, Switzerland). Samples of rumen fluid were taken once in period 1 and twice in period 2 and 3 for short chain fatty acids (SCFA) analysis. Data were acquired for pH in period 1, 2 and 3 and for RT in period 2 and 3. Data were analyzed by using the MIXED procedure of SAS 9.4. Data for variables obtained in period 1 served as a covariate. Values are represented as LS-Means ± standard error (SE).

Results: Glyphosate contamination did not influence RT, the daily average pH value and the concentration of total SCFA as well as the molar proportions of SCFA in the rumen fluid (Table 1). No evidence for subacute ruminal acidosis (average daily pH < 6.2; pH < 5.8 more than 324 minutes per day (2)) was identified. Only the period showed an effect on the pH-value and the SCFA, but no influence on RT (Table 1).

Table 1: Effect of glyphosate residues on pH-value, short chain fatty acids (SCFA) and rumination time (RT) of dairy cows (LS-Means±SE).

	Treatment		p-value		
	CON (n = 5)	GLY (n = 5)	group	period	group*period
RT [min/d]	593 ± 19	597 ± 19	0.898	0.736	0.706
pH [daily average pH]	6.4 ± 0.1	6.5 ± 0.1	0.415	0.004	0.117
pH < 5.8 [min/d]	55 ± 50	112 ± 50	0.453	0.089	0.178
Total SCFA [mmol/L]	49.1 ± 4.2	57.6 ± 4.2	0.207	0.121	0.235
Acetate [mol%]	68.8 ± 0.4	68.0 ± 0.4	0.210		0.388
Propionate [mol%]	17.0 ± 0.5	17.2 ± 0.5	0.732	0.003	0.831
Butyrate [mol%]	11.4 ± 0.3	11.2 ± 0.3	0.674	0.022	0.793

Conclusion: Present results suggest that a glyphosate contamination of feedstuffs did not influence rumen fermentation characteristics. Rumen microbiome will be analyzed to investigate possible specific treatment-related effects on ruminal microbial diversity.

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85.

Evaluating factors affecting the accuracy of determination of silage particle size using a forage particle separator

Untersuchungen zum Einfluss auf die Genauigkeit der Bestimmung der Partikelgrößen von Silagen unter Verwendung einer Schüttelbox

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Dairy cows require a diet with a sufficient proportion of forages rich in physically effective neutral detergent fibre (peNDF) to reduce the risk of subacute rumen acidosis (1). The peNDF is the product of the NDF content and the physical effectiveness factor, determined using a manually operated forage particle separator with different sieve sizes. However, several factors can influence the accuracy of the particle size measurement. The aim of the study was to evaluate the influence of single vs. varying operators and dry matter (DM) content on the sample's particle size distribution.

Methods: Grass and corn silage (40.3 and 28.6% DM, resp.) were collected from a conventional farm. Measurement of particle size distribution followed recommendations of a commercial forage particle separator (Shaky 4.0, Wasserbauer, Waldneukirchen, Austria), consisting of three sieves with hole diameters of 19, 8 and 4 mm (modification of [2]) besides a collection tray for particles < 4 mm. Approximately 1.5 L of silage were spread on the top sieve (19 mm) and the whole sieve set was shaken horizontally 12 times (forward and backward motion) at approximately 1.1 Hz, rotated by 90° and shaken again for all four directions of the sieve set (1). After removing the top sieve, the process was repeated in the same manner with only 4 shakes per direction for the 8 and 4 mm sieves. Repeated (n=10) shaking of one operator, several operators (n=10) and different DM contents of the silage (n=3, DM either adjusted by remoistening or drying to approx. 30, 35 and 40%) were tested as possible influencing factors. Data were subjected to ANOVA (SPSS 20.0) followed by SNK test.

Results: There was a high influence of the operator on the particle sizes' distribution regarding all sieves in both grass and corn silage (P<0.001), whereas the highest sample amount with the lowest standard deviation remained on the 19 and 8 mm sieve for grass and corn silage, respectively. Differences between operators were determined for all sieves in grass silage (P<0.001), but only for the 4 mm sieve in corn silage (P<0.001). The effect of DM on the particle size distribution (as % of the total sample weight per sieve) in grass and corn silage is shown in the following table:

	Grass silage					Corn silage				
Particle size (mm)	DM (%)			pooled sd	P	DM (%)			pooled sd	P
	30.5	35.5	40.3			30.5	34.7	42.8		
> 19	78.2	73.9	75.9	6.66	0.74	0.4	0.3	0.4	0.37	0.79
> 8	19.3^{a}	15.0^{a}	5.5^{b}	2.77	<0.001	44.3	37.3	26.2	8.73	0.11
> 4	1.6^{b}	7.3^{a}	9.4^{a}	2.44	0.001	42.6	44.8	51.8	7.04	0.32
< 4	0.9^{b}	3.8^{b}	9.1^{a}	2.53	0.001	12.7^{c}	17.6^{b}	21.7^{a}	1.69	0.002

Means in the same row with different superscripts differ significantly within silage (P<0.05).

Conclusions: Although there is a clear influence of the operator on particle size measurement, it can possibly be reduced by training as a certain learning effect in consecutive runs was observed. Handling the forage particle separator by varying operators is not recommended due to the fact that the highest sample proportion on one sieve size exceeded the smallest one by a multitude and therefore single results diverged widely. Silage DM affected the particle size distribution in both silages with the exception of particles > 19 mm, whereas a DM increase always resulted in an increase of the < 4 mm particle fraction, suggesting that small particles are less attached to larger particles in dry silages. Presumably, there is a substrate dependent effect as differences between DM levels were generally lower in corn silages. Hence, it can be expected that variations in total mixed rations with usually a high proportion of corn silage are lower.

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86.

Effect of dietary beta-carotene supplementation during the transition phase on colostrum quality of cows and incidence of diarrhea in calves

Effekt einer alimentären Supplementierung von Beta-Carotin während der Transitfütterung von Kühen auf Kolostrumqualität und Durchfallinzidenz von Kälbern

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Beta-carotene is assumed to have positive effects on the fertility of cows and also to improve the immunity system. Unfortunately, beta-carotene is subject to massive degradation during preservation and storage of feeds. As cows are often fed with such feedstuffs, particularly during the dry period, their beta-carotene intake might be quite low. Supposing that an alimentary supplementation of beta-carotene improves beta-carotene status of dairy cows, a feeding trial with pregnant cows was conducted with major focus on colostrum quality and health of calves.

Methods: The study involved 102 multiparous dairy cows supplied with beta-carotene in two levels (0 vs. 1000 mg per head and day) during 3 to 4 weeks before calculated date of parturition. Animals were fed a total mixed ration based on corn silage, brewer's grain, and wheat straw (intake of native beta-carotene: 280 mg per cow and day). Blood samples of cows were derived in weekly intervals and colostrum was collected after parturition in 14 cows per group. Newborn calves were supplied with colostrum of their mothers. They were housed and fed uniformly irrespective of previous treatments. Blood samples of calves were derived directly after birth (n = 14 per group) as well as the next day after consumption of colostrum (n = 15 per group). Incidence of diarrhea (liquid feces) was recorded for the following 2 and 4 weeks (male and female calves, resp.). Data analysis included one-way ANOVA and chi-square-test for diarrhea frequency.

Results: Dietary beta-carotene supplementation increased beta-carotene concentration in blood serum and colostrum of cows. Also colostrum retinol, total serum protein as well as gamma globulins were numerically higher. In newborn calves, levels of beta-carotene in blood serum were low irrespective of treatment but tended to be increased after consumption of colostrum from cows supplied with beta-carotene before calving. The same applied to total serum protein and gamma globulins in blood. In these calves, incidence of diarrhea was significantly reduced.

Table: Beta-carotene, serum protein, and gamma globulin concentration in colostrum and in blood of cows and calves and number of calves with diarrhea (means±SEM)

Beta-carotene supplementation of cows 3 weeks a.p., mg/d		**0**	**1000**	**P <**
Blood serum of cows a.p.	beta-carotene, mg/L	2.2 ± 0.5	3.2 ± 1.4	0.01
Colostrum	beta-carotene, mg/L	1.1 ± 0.7	1.9 ± 0.7	0.01
	free retinol, U/L	113 ± 23	138 ± 69	0.23
	total serum protein, g/L	156 ± 49	174 ± 49	0.36
	gamma globulin, g/L	104 ± 40	121 ± 41	0.28
Blood serum of calves at birth	beta-carotene, mg/L	0.6 ± 0.3	0.7 ± 0.2	0.63
Blood serum of calves after consumption of colostrum (day 1)	beta-carotene, mg/L	1.4 ± 1.4	2.5 ± 1.7	0.13
	total serum protein, g/L	57 ± 6	62 ± 9	0.10
	gamma globulins, g/L	15 ± 5	20 ± 10	0.08
Calves with diarrhea	out of 51 or 50, resp.	38	24	0.01

Conclusion: Beta-carotene supplementation during transition feeding of dry dairy cows improved colostrum quality which in turn reduced incidence of diarrhea in calves.

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87.

Hypoglycin A in bodyfluids of horses with atypical myopathy and their cograzing partners

Hypoglycin A in Körperflüssigkeiten bei Pferden mit atypischer Myopathie und deren Weidepartner

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Hypoglycin A (HGA) in seeds of *Acer pseudoplatanus* (Sycamore maple tree; SM) is suspected to cause atypical myopathy (AM), a fatal disease in horses on pasture (1 - 3). The aetiopathology is described as an acquired enzymatic deficiency of multiple acyl-CoA dehydrogenases, a highly fatal form of non-exertional rhabdomyolysis. Consequences are excessive myofiber lipid storage and abnormal high production of acylcarnitines which can be detected in bodyfluids besides the HGA content. The correlation of seed HGA content with the concentrations of HGA and toxic metabolites (MCPA-conjugates) in bodyfluids of affected horses was substantiated (1 - 3). Thus, cograzing partners exhibited no disease symptoms have not been studied alongside affected horses. Aim of the study was to compare HGA contents in bodyfluids of AM horses and cograzing, healthy (CH) horses on affected pastures to get information about the HGA status and the potential risk of developing AM.

Methods: We got knowledge from 16 diseased horses (7 Warmbloods, 4 Haflinger, 5 ponies comprising of 6 geldings, 3 stallions, 7 mares; 1.5 - 16 years old; BCS 5.0 - 5.5/9) on pastures in Germany with 6 - 24 h pasture turnout per day, which exhibited acute clinical signs of muscle pain and weakness. 15 horses died within 1 or 2 days after showing first clinical signs or were euthanized, 1 horse survived. In every case, AM was diagnosed by the veterinarian in charge based on the clinical signs and biochemical results. The AM horses originated from 11 pastures. Nine pastures were visited, SM seeds were taken and analyzed for HGA by LC-ESI-MS/MS. CH horses, which were present on AM affected pastures, were also investigated. Urine and blood serum samples from affected horses (serum = 8, urine = 6), their CH partners (serum = 12, urine = 4) and control horses without symptoms and contact to *Acer spp.* (serum = 5, urine = 4) were analyzed for HGA and MCPA-conjugates (UPLC-MS/MS). A SAS® based macro was used for statistical analysis.

Results: SM seeds were present on all pastures (1.7 - 319.8 µg HGA/g seed) with HGA contents similar to previous studies. The content of HGA in serum of AM horses ranged from 388 - 8,494 µg/L (controls < 10 µg/L; $P < 0.001$), and in urine from 144 - 926 µg/L (controls < 10 µg/L; $P < 0.001$), respectively. In AM horses the range of MCPA-carnitine in serum was 0.17 - 0.65 mmol/L (controls < 0.01) and in urine 0.34 - 2.05 µmol/mmol creatinine (controls < 0.001). CH horses showed higher concentrations of HGA in serum (109 ± 83.8 µg /L) and urine (27 ± 7.4 µg /L) compared to controls ($P > 0.05$), but lower concentrations compared to AM horses ($P < 0.05$). MCPA-glycine levels in urine of CH horses were higher in comparison to controls.

Conclusion: In AM horses, HGA intoxication was confirmed by the presence of HGA in SM seeds as well as high concentrations of HGA and MCPA carnitines in urine and serum. Cograzing, healthy horses did also ingest HGA containing material, as it is indicated by moderate HGA concentrations in blood and urine here, but they were obviously not exposed to a poisonous level. Consequently, toxic metabolites in bodyfluids were either not present (blood) or limited in terms of quantity and range (urine). Early detection of HGA in cograzing horses might be a promising prophylactic step (3).

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88.

Immunohistochemical localization of zinc sensing receptors in close proximity to CD3$^+$ T-cells in the pig intestinal tract

Immunhistochemischer Nachweis von Zink-sensitiven Rezeptoren in unmittelbarer Nähe von CD3$^+$ T-Zellen im Intestinaltrakt des Schweins

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Zinc plays a pivotal role in cellular metabolism and in gut health, which are affected by different cellular components. One of them, GPR39, a zinc sensitive receptor, which belongs to the ghrelin receptor family has been found to be involved in several cellular processes, such as cell survival, renewal, proliferation, differentiation, wound healing and neuronal signal transduction (1). Furthermore, three different subtypes of this receptor exist, which are denoted as LYPD1, GPR39-1a and GPR39-1b (2). All three forms are expressed in different tissues and in different quantities (2). High expressions of GPR39-1a, a full length form of the zinc sensing receptor, have been detected in liver, pancreas, kidney, adipose tissue and throughout the digestive tract (2). Studies on knockout rats have shown for the gastrointestinal tract that zinc sensing receptors were expressed in parietal cells in the stomach, in neurons within the plexus and in enterocytes lining the villi in the intestinal tract (3). The purpose of this study was to verify, whether the zinc sensing receptor was also expressed in the pig intestinal tract as described for rats.

Methods: The intestinal tract of six pigs (German Landrace) ranging in age from 1 to 7 weeks were used for immunohistochemical studies. Samples were taken from duodenum, jejunum, ileum and colon and were immersion-fixed in Bouin's solution. For cell-specific localization studies 4 µm thick and deparaffinized sections of all taken samples were incubated with an antibody against GPR39 and visualized by the avidin biotin peroxidase complex method with 3,3'-diaminobenzidine as chromogen. Additionally, double-immunostaining studies were performed with antibodies against GPR39, RP-11 and CD3 to clarify which lymphocyte cell type is in close proximity to GPR39 expressing cells.

Results: We found numerous GPR39 expressing cells solitary in the epithelial layer of villi and crypts throughout the pig intestinal tract in all ages investigated. Remarkably, the morphological structure of GPR39 expressing cells revealed columnar and slight forms which resembled those cells previously denoted as Lenten cells. Moreover, we also observed numerous lymphocytes in close proximity to GPR39 expressing cells, which were identified by double-immunostaining as T-lymphocytes.

Conclusion: Our results indicate that GPR39 possibly exerts an additional cellular function to those as yet described for this receptor and in the intestinal tract of pigs may be function as a mediator between extracellular zinc concentration and T-cell activation.

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89.

Postprandial kinetics of bacterial ecology parameters in the terminal ileum of pigs fed soybean meal or differentially processed blue sweet lupins

Postprandiale Kinetik der Parameter bakterieller Ökologie im terminalen Ileum von Schweinen die mit Soja oder unterschiedlich behandeltene Lupinen gefüttert wurden

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Question: Recent studies revealed that grinding intensity and hydrothermal processing might improve the nutritional value of blue sweet lupins (BSL, 1). Dietary particle size and digesta viscosity may change gastric emptying in the pig (2). Little is yet known whether this may influence small intestinal microbial ecology patterns during the postprandial phase. In the current study, postprandial kinetics of bacterial ecology was studied in growing pigs fed differentially processed BSL.

Methods: BSL were processed in a hammer mill passing either a 3 mm sieve (coarsely ground blue lupins; CBL), a 1 mm sieve (finely ground blue lupins; FBL), or ground to pass a 1 mm sieve and subsequently expanded in a modified single screw extrusion-cooker TS-45 (ZMCh Metalchem Gliwice, PL) at 110 to 120 °C (expanded blue lupins, EBL). Four experimental diets were formulated based on wheat and barley and either soybean meal or BSL as main protein source. A soybean meal-based diet (SBM) served as control and had similar particle size distribution as the EBL diet. Twelve PIC x Danbred crossbreed pigs with an initial body weight of 20 kg were surgically fitted with a simple T-cannula at the terminal ileum and offered the experimental diets twice daily in mash form in a 3 x 4 Latin square design. After a 7-day adaptation period, ileal digesta samples were taken every 2 h over the course of 12 h after the morning meal. Microbial metabolites D-/L-lactate, SCFA, NH3 were determined. Total DNA and RNA were extracted from ileal digesta using commercially available kits. The 16S ribosomal DNA and RNA copy numbers were determined by qPCR (RT-qPCR) using primers specific for lactobacilli, enterobacteria, *Bacteroides-Porphyromonas-Prevotella*, and three clostridial clusters. Data analyses were conducted using SPSS.

Results: Both time point and dietary treatment had statistically significant effects on bacterial metabolites and bacterial DNA and RNA copy numbers. Concentration of SCFA in ileal digesta increased ($P<0.05$) with SBM and EBL and peaked (20 and 23 mmol/L, respectively) after 4 hours, whereas CBL and FBL diets showed only minor effects on SCFA concentration (maximum 9 and 7 mmol/L, respectively). Similar patterns were observed for individual SCFA, although concentration of propionate and butyrate were generally low compared to acetate. In contrast, total lactate was highest (90 mmol/L; $P<0.05$) after 4 hours in CBL-fed pigs, whereas SBM (56 mmol/L) and FBL (54 mmol/L) showed peaks after 6 hours, and EBL diets peaked (76 mmol/L) at 8 hours after the morning meal. Area under the curve (AUC) values were used for all parameters and correlated with digestibility data of proximate nutrients. The amount of undigested starch was positively associated with total lactate ($R = 0.38$), enterobacterial 16S rRNA gene copies ($R = 0.47$) and clostridial cluster XIVa ($R = 0.41$), whereas undigested protein was positively correlated with the abundance of clostridial cluster IV. Total SCFA were positively associated with intake of dietary particles <0.4 mm, and negatively associated with particles >1.0 mm.

Conclusions: The results revealed clear differences in postprandial kinetics of bacterial metabolites and 16S rRNA copy numbers in the ileum of pigs fed diets containing differentially processed BSL. These differences seem to be related to particle size and digestibility of nutrients. Further analyses will reveal whether digesta transit has additional effects. The data also highlight that the choice of sampling time point is crucial for interpretation of microbial ecology in digesta contents.

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90.

Histological changes of the jejunal wall in elongated small intestine resulting due to an exocrine pancreatic insufficiency (EPI) in pigs

Histologische Veränderungen in der Darmwand von Schweinen bei einer Dünndarmelongation infolge einer exokrinen Pankreasinsuffizienz (EPI)

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Exocrine pancreatic insufficiency (EPI) in pigs is induced through a pancreatic duct ligation (PL) and serves as a model for EPI in humans. In former studies (1, 2) an elongation of the small intestine (SI) in pigs with an EPI up to 6 meters within 10 weeks was observed. Besides high serum levels of GLP-2, a higher proportion of butyrate and propionate were measured in ileal chyme (1, 2) of PL-pigs. In addition a mucosal atrophy was found in the SI of PL-pigs due to the lack of pancreatic juice by (3). This study aimed to examine histological changes in the architecture of the SI from PL-pigs compared with healthy ones to gain more information about the mechanisms behind the SI elongation.

Methods: The tissue samples derived from a previous study (1): 19 pigs were separated into 4 groups, one control-group (C, n=4) and 3 groups of PL-pigs. The C-group underwent a sham-op when aged 7 weeks. Ten pigs underwent PL-surgery at life week 7 (PL7) and 5 at life week 16 (PL16, n=5). All pigs were fed a complete diet, 5 pigs of the PL-7-group were fed additionally a vitamin additive (90,000 IU Vit A/kg dm diet, 600 mg Vit E/kg dm diet) from week 9 onwards (PL7 VIT, n=5). All pigs were euthanized and dissected at the age of 26 weeks. Tissue samples were taken every two meters of the SI. Only samples from the proximal, middle and distal jejunum were analysed. They were cut, dehydrated and embedded in paraffin. 5µm sections were stained with hematoxylin and eosin. In each section only well oriented villi were measured. Jejunum mucosal height is defined as sum of crypt depth + villus height. In addition immunohistological staining was performed: Antiserum directed against PCNA (proliferating-cell-nuclear-antigen) was used to determine proliferation rate, which was described as percentage of PCNA-positive cells per cryptcell number.

Results: The PL7 VIT group showed an increase in mucosal height compared to the C-group. The mucosal proliferation rate was higher in the PL7 VIT and PL16 group compared to C-group (table 1).

Table 1:Small intestine length and mucosal parameters measured at the age of 26 weeks (mean of samples from the proximal, middle and distal part of the jejunum).

	Control			PL7			PL7 VIT			PL16		
Length of SI (m)*	18.3^{a}	±	1.33	19.8^{ab}	±	4.50	24.3^{b}	±	1.55	24.6^{b}	±	2.40
Proliferation rate (%)	25.5^{a}	±	4.76	29.4^{a}	±	7.60	37.3^{b}	±	9.13	36.3^{b}	±	11.0
Mucosal height (µm)	703^{a}	±	208	721^{a}	±	191	831^{b}	±	190	692^{a}	±	167
Crypt depth : villus height ratio	1:2.04	±	0.76	1:2.36	±	0.96	1:2.32	±	1.23	1:1.94	±	0.70

Different letters (a,b) mark significant ($p<0.05$) effects of group. *data from MÖSSELER et al. 2015

Conclusion: The results indicate that the mucosal proliferation rate was higher in groups in which the elongation of the SI was highest (PL7 VIT, PL16). These effects were accompanied by an increased amount of undigested nutrients and higher short-chain fatty acid (SCFA) levels in the digesta of the distal SI (1). Both can be a trigger for enteroendocrine L-Cells to produce more GLP-2 (4), which would be consonant with the higher GLP-2 serum levels in PL-pigs (1). As GLP-2 is known to stimulate mucosal growth (5) the finding of highest GLP-2 levels in PL7 VIT might explain the increased mucosal height in that group. It can be speculated that the higher GLP-2 levels in that group are resulting from a higher feed intake rate.

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91.

Kinetic characteristics of Lipopolysaccharide-induced immune response in chicken

Kinetische Charakteristika einer Lipopolysaccharid induzierten Immunantwort in Hühnern

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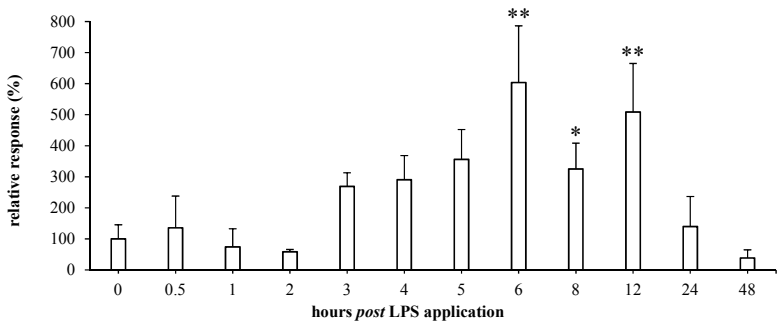
The aim of the present study was to identify suitable immunologic and hematologic parameters and relevant points in time indicative for an inflammatory response of laying hens in a lipopolysaccharide (LPS) challenge model. In additional future work to (1), different genotypes of laying hens will be compared for their immune responses under nutritive stress conditions. Therefore, we investigated the time course of the inflammatory response in laying hens triggered by exposure to LPS.

Methods: Innate immune reaction in 49-week old Lohmann Brown hens (n=36) was induced by intramuscular application of 2 mg LPS (*Escherichia coli* 0111:B4)/kg body weight. Blood was taken directly after slaughtering at different points in time (0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24 and 48 h) and was analyzed for total leukocytes counts and differential cell counts. For evaluation of the redox response the oxidative damage was investigated in plasma via lipid peroxidation, total antioxidative capacity and the production of nitrogen oxides. Clinical characteristics were recorded frequently for 7 h *post* LPS. ONEWAY ANOVA analysis was performed for the factor time using STATISTICA.

Results: Leukopenia started after 0.5 h with lowest value at 3 h, and returned to base levels after 5 h with a second decrease at 8 and 12 h due to heterophilia reaching highest value at 5 to 6 h and returning to initial levels then. Lymphopenia occurred after 3 h and started returning to initial levels from 12 h *post* LPS. Clinical symptoms such as ruffled feathers, reduced activity up to somnolence, and anemic wattles occurred within 6 h *post* LPS. Body temperature decreased within 2 h from initially 41.1 °C to 40.4 °C at 2 h, and returned to initial value. Antioxidative capacity was transiently decreased at 2 h to 5 h and then returned to base levels. Lipid peroxidation decreased from 1 h *post* challenge and remained low (both no significance). Nitrite concentrations in blood plasma (Figure 1) increased within 6 h *post* LPS up to 600 % of initial value of 7.6 µM at 0 h.

Figure 1 Change in nitrite (NO_2) concentrations in blood plasma relative to starting point over time. Columns represent means (+ SD) of 3 animals at each time. (Dunnett *post hoc* test * p≤0.05; ** p≤0.001)



Conclusion: Maximal immune response of adult laying hens was detected within 6 h *post* LPS challenge for most of the investigated parameters. Analysis of white blood cells and recording of clinical symptoms are useful tools to display the course of inflammatory response in chicken. Determination of nitrite and antioxidative capacity in blood plasma seem to be suitable parameters to show redox response after immune stimulation, while under given conditions lipid peroxidation seems to be not. These findings will determine the time course for the main trial.

Lieboldt MA, Halle I, Frahm J, Schrader L, Weigend S, Preisinger R and Dänicke S (2015): J Poult. Sci., published online, DOI: 10.2141/jpsa.0150067

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92.

Effects of general anesthesia with propofol, pentobarbital or isoflurane plus propofol treatments on plasma metabolites and hormones in pig

Wirkungen einer Vollnarkose mit Propofol, Pentobarbital oder Isofluran plus Propofol auf Metaboliten und Hormone im Plasma beim Schwein

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Experimental setups for physiological studies, in which acute operative interventions need to be performed (e.g. measuring blood flow in uterus of pregnant animals), require inclusion of sedation and/or general anesthesia, which may interfere or confound with the effects of the experimental factors of interest on measured variables. We have recently shown that the most commonly used sedatives/anesthetics in pigs (e.g. ketamine, xylazine, azaperone) affect acute physiological responses of pigs and thus the primary metabolic readouts have the potential to be confounded (1). To extend the search for a physiologically-friendly anaesthesia regime for such studies, we investigated effects of general anaesthesia induced by propofol (**Prop**) or pentobarbital (**Pent**) or propofol plus isoflurane (**Prop + Isof**) on plasma concentrations of commonly measured metabolites and hormones.

Material and methods: In two experimental repetitions, 6 female pigs (118 ± 9.8 kg) fitted with jugular vein catheters were used. On the first and last experimental days (ED: 1-12 d) basal measurements were made on plasma samples collected at regular intervals for a period of 6 h (**CTRL**). On the following EDs, each pig was rotationally anaesthetized either with Prop or Pent or Prop + Isof on different days, separated with washout periods of sufficient length (2-3 d). Plasma concentrations of glucose, lactate, NEFA, triglycerides (TG), cholesterol, urea as well as hormones including glucagon, insulin and cortisol were determined by spectrophotometry, RIA, and ELISA, respectively. Data were analysed with repeated measures ANOVA using a mixed model, and post-hoc group comparisons with the Tukey-Kramer test.

Results: Plasma concentrations of cholesterol, urea, glucagon and cortisol remained unaffected by any of the treatments (data not shown; P>0.05) with no significant interaction by time (P>0.05). Glucose and lactate concentrations were increased (Table 1; P

Table 1 Effects of the examined anesthetics on selected plasma metabolite and hormone concentrations in pigs.

Item	CTRL	Prop	Pent	Prop + Isof	SE	P-values		
						Trt	Time	Int.
Glucose, mmol/L	4.11^{ab}	4.61^{ab}	5.18^{a}	3.91^{b}	0.333	0.038	0.001	0.001
Lactate, mmol/L	0.39^{a}	0.83^{b}	1.22^{c}	0.54^{a}	0.077	0.001	0.001	0.016
NEFA, µmol/L	373^{a}	226^{b}	210^{b}	327^{ab}	40.5	0.018	0.001	0.014
TG, µmol/L	289^{a*}	309^{ab}	287^{a}	319^{b*}	9.0	0.020	0.001	0.001
Insulin, ng/mL	1.32^{a}	1.60^{b}	1.49^{ab}	1.50^{ab}	0.057	0.013	0.001	0.011

CTRL: Control; **Prop:** Propofol**; Pent:** Pentobarbital**; Prop + Isof:** Propofol + Isoflurane; **SE**: The largest standard error of the LSMEANS; **Trt**: Treatment; **Int**.: Interaction; **abc**: Different letters on the same line indicate significant (P*: Indicates tendency (P

Conclusions: Our data demonstrate altered levels of metabolites and hormones induced by all three treatments with propofol plus isoflurane exerting less pronounced effects, suggesting varying degree of alterations in kinetics of carbohydrate and lipid metabolism induced by anaesthesia. Nevertheless, as compared to azaperone and xylazine the effects of Prop, Pent and particularly Prop + Isof on metabolite concentrations are much smaller.

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93.

Effect of an extra vitamin supply in young pigs with experimentally induced pancreatic exocrine insufficiency on body composition characterized by analysis of the hind leg with MRI technique

Einfluss einer deutlich bedarfsüberschreitenden Vitaminsubstitution bei jungen Schweinen mit experimentell induzierter exokriner Pankreasinsuffizienz auf die Körperzusammensetzung - charakterisiert mittels MRT Untersuchungen der Hintergliedmaße

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The maldigestion of nutrients caused by exocrine pancreatic insufficiency (EPI) results in growth retardation in juveniles not treated with a pancreatic enzyme replacement therapy (PERT). The pancreatic duct ligated (PL) pig is an established model to study EPI. As a marked increase of relative weight of the gastrointestinal tract (GIT) was observed in PL-pigs [1], the question arose, whether the body composition (bc) of the empty body (without GIT) is affected as well. The aim of this study was to measure body size and bc in pigs with or without induced EPI. The question whether the increased growth induced by vitamin supplementation affects bc was of special interest.

Material and Methods: A total of 14 piglets were used in this study. PL was performed in 10 pigs at the age of 7 weeks (PL 7). 5 of the PL 7 pigs received extra oral vitamin supply (PL 7 + V). No PERT was given to any PL-pig. A complete diet was fed (pair feeding; last two weeks: ad lib. feeding). Animals were euthanized at the age of 26 weeks and dissected. Total GIT was removed to determine the empty bw (ebw). To quantify the muscle volume, the fat content within the muscle and the ratio between fat to muscle of the hind leg MRI analysis was performed (1.5Tesla device; Avanto, Siemens Healthcare). Leptin was determined in serum by use of a porcine specific Leptin ELISA (Sea084Po, cloud-clone corp., Houston, USA) in serum samples taken at day of dissection. Statistical analysis was done by GLM procedure, post-hoc test: Fisher`s LSD test using SAS®.

Results: EPI caused a significant reduction in bw, mass and volume of the hind leg and an increased relative weight of the GIT (see table 1). While fat content of the muscle and ratio of fat:muscle tended to be lower in PL-pigs, the ratio muscle:ebw and muscle:hind leg was higher in PL-pigs. The vitamin supplementation resulted in increased values for all body parameters; there was also a trend for a higher fat content in muscle and higher leptin levels in serum in PL 7 + V.

Table 1: Body weight, body length, empty body weight, volume of hind leg, volume of muscle, fat content in muscle, ratio fat to muscle of control pigs and PL-pigs - measured at the age of 26 weeks

	Control (n=4)	PL 7 (n=5)	PL 7 + V (n=5)
Body weight (kg)	112 ± 9.72 a	45.6 ± 30.2 c	75.4 ± 13.8 b
Empty body weight (kg)	103 ± 8.25 a	38.0 ± 26.0 c	62.3 ± 11.8 b
Rel. weight of GIT (% of bw)	7.89 ± 0.940 a	17.3 ± 2.02 b	17.4 ± 1.32 b
Mass of hind leg (kg)	10.2 ± 0.194 a	3.95 ± 2.63 b	6.05 ± 1.44 b
Volume of hind leg (cm^3)	9455 ± 309 a	4041 ± 2679 b	6038 ± 1612 b
Volume of muscle (cm^3)	7072 ± 1032 a	3274 ± 2042 b	4693 ± 968 b
Fat content in the muscle (%)	6.57 ± 1.26 a	5.32 ± 1.17 a	6.14 ± 1.41 a
Ratio of fat to muscle* (%)	25.3 ± 9.42 a	17.5 ± 3.40 a	20.9 ± 7.54 a
Muscle / kg ebw (cm^3 / kg)	92.0 ± 9.53 a	107 ± 6.46 b	95.9 ± 13.2 a
Muscle / hind leg (Vol %)	74.7 ± 9.43 a	82.5 ± 3.40 a	9.1 ± 7.54 a
Serum leptin (ng/ml)	14.9 ± 11.8 a	0.386 ± 0.262 b	1.02 ± 1.30 b

Different letters mark significant effects of treatment ($p < 0.05$).

Discussion: As described before [1; 2] proportion of GIT was higher in PL-pigs. By using MRI it was possible to characterise the bc more in detail. There was a trend for a lower fat content in the muscle and a lower ratio of fat:muscle in PL 7 indicating effects of EPI on bc which is also indicated by the leptin levels. Even the statistical significance was not reached due to the low number of animals and individual variation, the marked effect of extra vitamin supplementation is noteworthy under clinical aspects. The results indicate that the MRI analysis of bc is a valuable method to detect changes in bc in clinical patients as well as in experimental studies as it allows a deeper understanding of changes occurring in case of maldigestion and malnutrition. *References available on request*.

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94.

Impact of dietary concepts on natural *Lawsonia intracellularis* infections in finishing boars

Einfluss unterschiedlicher Fütterungskonzepte auf eine natürliche Lawsonia intracellularis Infektion in der Ebermast

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Question: *Lawsonia intracellularis (L.intracellularis)* is one of the economically most important pathogens in the swine production worldwide (1). Up to now, there are only small numbers of studies concerning potential dietary effects on *L. intracellularis* infection in pigs (1; 2). The acute and most severe form is commonly found in high health herds when replacement gilts and boars have been introduced into a new farm site (3). This study tested the hypothesis whether the composition of diets for fattening boars has an impact on the amount of excretion of *L. intracellularis* in a natural infection model.

Methods: Genetically defined boars (n=50; ~90 kg BW) from a specific pathogen free farm were on site blood sampled and serologically tested for antibodies against *L. intracellularis.* After that, boars were allotted to five dietary treatments with subgroups of five animals each. Each group was fed one of five differently processed but isoenergetic and isonitrogenous diets ad libitum: a finely ground pelleted diet (FP), a coarsely ground meal diet (CM), a usually ground meal diet either with 22 % cracked corn (CORN), 16.9 % dried whey (WHEY) or 30 % raw potato starch (RPS). During the last four weeks before slaughter, faecal samples were taken once a week to analyse the counts of *L. intracellulars* in faeces by qPCR. Thereafter, the boars were slaughtered and caecum content was taken for qPCR, caecum wall for histological analyses. Blood samples were analysed serologically for *L. intracellularis* status (PI values in a blocking ELISA). Performance parameters have also been recorded. Statistical analysis was performed with SAS for Windows, procedure GLM (LSD-Test) or Wilcoxon-Test.

Results: In this experiment, there were no significant differences between the groups in terms of the results of serological investigations and counts of *L. intracellularis* in faeces. At start, 50 % of animals were serologically positive. At slaughter, 98 % of the samples were serologically positive. The group, receiving dried whey in the diet had the significantly lowest number of *L. intracellularis* genome equivalents in caecal content. In RPS-groups, the numerically highest *L. intracellularis* excretion, a significantly higher crypt depth, the significantly lowest dry matter content in the faeces and an unfavourable performance on an overall high level existed.

Table 1: Results of serological tests, counts of *L. intracellularis* (L.i.) genome equivalents (GE) and histological examinations as well as DM in faeces and performance parameters

diet	FP	CM	CORN	WHEY	RPS
n	10	10	10	10	10
PI values blocking ELISA week 0	31.0^{a}±12.9	30.5^{a}±16.9	25.5^{a}±13.1	29.0^{a}±14.7	32.9^{a}±14.6
Δ PI values (slaughter minus week 0)	22.7^{a}±18.9	19.6^{a}±23.3	32.4^{a}±14.2	28.3^{a}±16.2	25.8^{a}±17.6
lg GE L.i. (/1 g faeces) week 0	2.46^{a}±2.64	3.58^{a}±2.54	3.43^{a}±2.37	2.30^{a}±3.16	2.58^{a}±2.73
lg GE L.i. (/1 g faeces) Ø week 1to4	3.40^{a}±1.53	3.01^{a}±1.41	3.80^{a}±1.72	3.98^{a}±2.20	4.08^{a}±2.13
Δ Δ lg GE L.i. (Ø week 1to4 - week 0)	0.93^{a}±3.13	-0.57^{a}±3.30	0.37^{a}±2.65	1.68^{a}±3.58	1.51^{a}±3.80
lg GE L.i. (/1 g caecum content)	4.34^{ab}±3.83	5.46^{a}±3.03	5.16^{a}±3.63	1.57^{b}±3.32	5.82^{a}±3.31
crypth depth caecum (µm)	482^{a}±57.5	473^{a}±50.0	499^{a}± 106	475^{a}±66.0	570^{b}± 189
DM faeces (g/kg faeces) Ø week 1to4	269^{a}±33.2	245^{ab}±15.2	258^{a}±29.6	243^{ab}±15.2	225^{b}±15.2
daily feed intake (g DM/kg bw)	26.8	28.4	28.1	29.0	25.2
daily body weight gain (g/d)	1326±273	1273±161	1258±131	1261±198	1104±336
feed conversion ratio (feed:gain)	2.64	2.87	2.93	2.94	2.94

Conclusions: Previous studies (1; 2) suggest a link between diet and colonization with *L. intracellularis* - higher number of lactobacilli in the small intestine, which may delay the colonization of *L. intracellularis*. The results of this study do not refute this hypothesis. Further studies are necessary to verify whether certain sugars (lactose- group WHEY) or starch sources (group RPS) interfere with processes in a *L. intracellularis* infection.

Parts of the project were supported by the Federal Ministry of Food and Agriculture, Germany.

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95.

Comparison of DNA- and 16S rRNA based quantification of the porcine intestinal microbiota

Vergleich von DNA und 16S rRNA basierter Quantifizierung der porcinen Mikrobiota

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Molecular methods have been established in the field of animal science as a superior means to study the impact of dietary changes in animals. However, most often assays to quantify bacterial 16S rDNA are used. As DNA based assays only show the bacterial potential, but not the actual metabolic activity, this study investigated the differences between rDNA- and rRNA based PCR assays and their impact on the analysis of the intestinal bacteria.

Methods: Quantitative assays were used to compare bacterial composition (qPCR of 16S rDNA) and ribosomal activity (rT-PCR of rRNA) in nucleic acid extracts. Lactobacilli, enterobacteria, three clostridial clusters as well as the *Bacteroides-Prevotella-Porphyromonas* cluster were used a model target bacteria of intestinal importance. To explore postprandial bacterial responses, ileal samples (n=11 per group) of fistulated fattening pigs fed soy bean or extruded blue sweet lupin supplemented diets were analyzed at 0,2,4,6,8,10 and 12h after feeding. Differences for intestinal sites (stomach, jejunum, colon ascendens) were studied in samples from 14d old suckling piglets (n=8 per group). The control group was left with their mothers, while animals of the treatment group were transferred to artificial rearing units and fed a milk replacer diet. Statistical analysis of the data was carried out by the Kruskal-Wallis test and Mann-Whitney-U test for direct comparison. Time dependent differences were analyzed via Friedman-test for paired samples; spearman correlation was used for the correlations between bacterial metabolites and rRNA/rDNA.

Results: Quantitative PCR of the 16S rDNA gene did not show pronounced differences after feeding for most bacterial groups except for moderate increases of ileal lactobacilli and the clostridial cluster XIVa. However, analysis of the ribosomal RNA showed a drastic increase in the amount of 16S rRNA for the same groups. Time dependent differences were non-significant for rDNA based assays in soy bean ($p=0.566$) or extruded lupins ($p=0.135$), but highly significant for rRNA content in the lupin diet ($p=0.002$). Based on rDNA analysis, only minor significant differences were found between soy bean- and lupin supplemented diets, contrary to rRNA based analysis of lactobacilli where significant differences were observed for almost all sampling times. Furthermore, for bacterial groups that showed no significant total changes of rRNA or rDNA, RNA to DNA ratios suggested an increase in ribosomal content. Finally, significant correlations (n = 35 per group) were found for bacterial metabolites and rRNA, but not for rDNA.

Regarding intestinal sites, moderate increases of rRNA and rDNA content occurred for lactic acid bacteria and enterobacteria throughout the intestine of suckling piglets. Ribosomal RNA and rDNA content of strict anaerobic bacterial groups increased more than tenfold in the hind gut. Contrary to fattening pigs, a significant impact of diets was observed for both rDNA and rRNA. However, significance differences were only achieved in the small, but not in the large intestine. Furthermore, the ratio of lactobacilli to the *Escherichia* group, often used as an indicator for intestinal health, was significantly different between treatments for rRNA based assays in all intestinal segments (stomach $p=0.015$; jejunum $p= 0.043$, colon = 0.038), but only significant in the colon for rDNA based assays (stomach $p= 0.130$, jejunum $p=0.505$, colon = 0.007). Contrary to fattening pigs, the correlation analysis (n= 30 per group) showed no clear correlation of bacterial metabolites to rRNA or rDNA, but rather significant correlations for both nucleic acid fractions. Finally, RNA/DNA ratios suggested that bacteria were much more active in the stomach and small intestine than in the hind gut.

Conclusions: The results show that rRNA based assays are much more sensitive regarding changes in bacterial metabolism. Differences between rRNA and rDNA content were observed for fattening pigs, but not for neonatal piglets, which may indicate that intrabacterial competition is not as pronounced in very young animals. For comparative studies targeting the activity of the intestinal microbiota, it is suggested to use rRNA based analysis instead of rDNA based assays. This will require only minor methodological changes in sample extraction and amplification.

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96.

Effects of concentrate type and concentrate-to-roughage ratio on protozoa numbers in the Hohenheim gas test

Auswirkungen der Kraftfutterart und des Kraftfutter-Raufutter-Verhältnisses auf die Protozoenanzahl im Hohenheimer Futterwerttest

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The Hohenheim gas test (HGT) is a widely used in vitro fermentation system to study rumen metabolism. The successful use of such a rumen simulation technique benefits much from the maintenance of the original microbial populations. Studies on the development of the microbial community in the HGT, in particular regarding the protozoa population, are scarce. The objectives of the present study were to investigate (i) the development of protozoa numbers in general and (ii) the impact of diet composition on this trait in the HGT.

Tab. 1: Chemical composition of the diets.

diet	CP	EE	aNDFom	starch	energy
	[g/kg DM]				[MJ ME/kg DM]
W 30:70	108.9	20.0	422.4	177.9	10.8
DC 30:70	141.9	22.5	505.7	49.9	10.6
W 70:30	119.4	19.7	249.2	467.2	11.9
DC 70:30	215.4	34.3	429.7	81.1	11.4

Methods: In a 2x2 factorial approach, two types of concentrate and two concentrate-to-roughage (C:R) ratios were tested. The concentrate types were wheat (W) and a commercial dairy concentrate (DC); C:R ratios were 30:70 and 70:30 (dry matter basis). Grass hay was used as a roughage source in the diets. The chemical composition of the diets is shown in Tab. 1. Each diet was tested in triplicate in two incubation trials, respectively, resulting in six replicates per diet and incubation time. Samples for the determination of protozoa numbers were taken after 0, 8, 24 and 48 hours of incubation. Protozoa were counted in a Fuchs-Rosenthal counting chamber and were differentiated into entodiniomorphs and holotrichs. Statistical analysis was performed using a linear mixed-effects model (fixed effects: concentrate type, C:R ratio, incubation time; random effect: trial).

Results: For all diets, total protozoa numbers increased within 24 hours of incubation and decreased thereafter (Fig. 1a). Wheat diets significantly increased ($p < 0.001$) total protozoa numbers, compared to DC diets. The opposite was observed for the holotrichs, which were significantly more abundant ($p = 0.03$) in DC diets (Fig. 1b). Neither total protozoa ($p = 0.45$), nor holotrichs ($p = 0.54$) were affected by the C:R ratio.

Conclusions: The results of the present study demonstrate that high numbers of protozoa can be maintained in HGT systems over an incubation period of 48 hours. This finding is contrary to previous results (1), where we observed a strong decrease of protozoa numbers. This might be due to a 3-fold lower initial protozoa concentration in the rumen fluid used in the present study, resulting in a higher supply of feed for the protozoa population. The positive effect of starch-rich diets on protozoa numbers in vivo, especially on entodiniomorphs, is a common finding and is supported by our results. The lacking effect of the concentrate level on protozoa numbers could be attributable to an excess of nutrients present in the concentrate-rich diets.

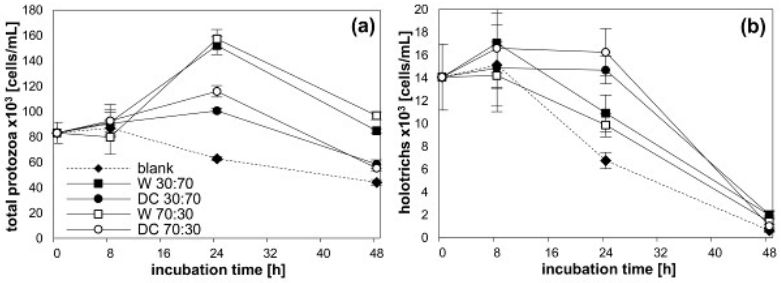

Fig. 1: Effects of concentrate type (W = wheat, DC = dairy concentrate) and concentrate-to-roughage ratio on **(a)** total protozoa and **(b)** holotrichs in the Hohenheim gas test (mean±SEM, n = 6).

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97.

Effect of water restriction on microbial biomass in goat faeces

Einfluß einer eingeschränkten Trinkwasseraufnahme auf die mikrobielle Biomasse im Kot von Ziegen

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Over the past five decades, water restriction in ruminants has been widely studied, mainly to evaluate and understand the physiological processes that enable ruminants to tolerate water shortage. Despite this extensive research, information considering the effect of water restriction on faecal composition is largely unexplored. Therefore the present study aimed to determine if and how water restriction changes the composition of microbial biomass in goat faeces.

Methods: A three-month trial set up as a complete Latin square design was carried out in Sultan Qaboos University, Oman, in 2014. Nine adult male goats were subjected to 3 watering regimes: 1) water offered *ad libitum* (control); 2) water restricted to 85% of individual *ad libitum* consumption; 3) water restricted to 70% of individual *ad libitum* consumption. The trial entailed 3 periods, each comprising 16 days of adaptation and 8 days of sampling. Rhodes grass hay and barley were fed at a ratio of 1:1 at 1.3 times maintenance energy requirement for metabolizable energy. On days 2, 4 and 6 about 30 g of fresh faeces were collected 1 hour after morning feeding and immediately frozen at -20 °C. The samples were then freeze-dried before analysis. Ergosterol was determined (Zelles, 1987) to assess fungal biomass in faeces. The analysis of amino sugars mannosamine, muramic acid, galactosamine and glucosamine (Indorf et al., 2011) served the determination of both fungi and bacterial biomass. The mixed model procedure in SAS was used to conduct ANOVA with period and treatment as fixed effects and animal as random effect.

Results: Water restriction had no effect on faecal concentrations of ergosterol, fungal C, mannosamine, galactosamine and glucosamine. Bacterial C and muramic acid were significantly reduced (p0.05), the mean values were higher in the control compared to the 70% treatment. Although the faecal concentrations of microbial C were not statistically different (p>0.05), the mean values were higher in the control compared to the 70% treatment.

Parameters (mg g^{-1} DM)	Water offer relative to ad libitum consumption (Means + SD)		
	70%	**85%**	**100%**
Microbial C	37.19+6.053	38.25+12.042	42.97+6.922
Fungal C	11.67+3.668	10.97+3.636	11.83 + 4.249
Bacterial C	24.45^{b}+ 5.030	27.07^{ab}+ 10.622	31.60^{a}+ 7.924
Fungal C/ Bacterial C ratio	0.52+0.179	0.45+0.199	0.42+0.243
Fungal C/ Microbial C ratio	0.34+0.073	0.29+0.092	0.28+0.103
Mannosamine	0.25+0.131	0.22+0.142	0.26+0.096
Muramic acid	0.54^{b}+ 0.106	0.60^{ab}+ 0.227	0.70^{a}+ 0.176
Galactosamine	1.56+0.387	1.59+0.413	1.57+0.340
Glucosamine	2.10+0.457	2.07+0.588	2.29+0.379
Fungal glucosamine	1.34 + 0.370	1.24 + 0.413	1.29 + 0.432
Ergosterol (µg g^{-1} DM)	1.81+0.481	1.50+0.525	1.87+0.603

Within rows, means with different superscript differ at p

Conclusions: Water restriction did not affect faecal fungal biomass but decreased bacterial biomass. Since faeces with stable fungi communities exhibit lower N emissions and stronger N immobilization, water restriction has implications for the availability of N to plants if such faeces are used as manure.

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98.

Effects of an oral inulin supplementation on trimethoprim/sulfadiazine induced changes of microbial metabolism in the equine caecum

Effekt einer oralen Inulin-Supplementierung auf Trimethoprim/Sulfadiazin induzierte Veränderungen des mikrobiellen Stoffwechsels im equinen Caecum

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Antimicrobial therapy in horses is always associated with potential disturbances in the intestinal microflora and may sporadically result in typhlocolitis. In order to minimize the risk of this severe side effect, prebiotics are often supplemented in line with the administration of antibiotics despite limited evidence-based data supporting their efficacy in this species. Trimethoprim/sulfadiazine is an antimicrobial widely used in equine practice. The aim of the present study was to determine the potentially protective effects of the prebiotic inulin on trimethoprim/sulfadiazine induced changes of microbial metabolism in the equine caecum using the colon simulation technique (COSITEC).

Animals and Methods:

Eight horses were included in the study, subdivided into two feeding groups and subjected to a standard feeding regime supplemented with either topinambur powder (inulin group) or corn cob meal (control group) for three weeks. Subsequently, caecal contents of all animals were harvested for in vitro studies using COSITEC. Two identical trials were conducted with 12 fermentation vessels over a period of 12 days consisting of a 5-day equilibration period followed by a 7-day experimental period. In each trial, six fermentation vessels were assigned to one of the two feeding groups. Caecal contents of two horses of each group served as substrate. During the experimental period, trimethoprim/sulfadiazine was administered into half of all fermenters of each group (25 mg/fermenter/day) for 7 days. The effects of inulin feeding and of antibiotics on pH, redox potential, total gas production, concentration of NH_3 and short chain fatty acids (SCFA) were determined. Data were analysed by Student's t test for unpaired observations or by 2-way repeated measures ANOVA with Bonferroni's post test. Significance was set at $p < 0.05$. Structure of microbial community was analysed by means of single strand conformation polymorphism (SSCP).

Results:

Mean baseline pH values and mean redox potentials during the experimental period were not significantly different between the inulin group (trial 1, 6.05±0.14 and 192±24 mV, respectively) and the control group (trial 1, 5.97±0.02 and 203±18 mV, respectively). The addition of trimethoprim/sulfadiazine led to an increase in pH ($p < 0.01$), to a decreases in digestibility of organic matter ($p < 0.01$) and to a decrease in production rate of total SCFA ($p < 0.05$) mediated by a decrease in proprionate and acetate production in both feeding groups. In the presence of sulfadiazine/trimethoprim, topinambur powder led to an increase in NH_3 production ($p < 0.05$). Total gas production was not affected. Effects on structure of microbial community were not detected.

Conclusions:

The present study clearly documents effects of trimethoprim/sulfadiazine on caecal microbial metabolism in horses. However, these changes were not abolished by feeding inulin suggesting that inulin may not be an appropriate prebiotic for preventing antibiotic-induced changes in the hind gut of horses.

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99.

A case report about hypervitaminosis D in alpacas

Hypervitaminose D in einem Alpaka-Bestand - ein Fallbericht

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Introduction: Alpacas with reduced body condition and partly severe general condition of a herd of 80 animals (stallions, mares, foals) attracted their owner's attention. The consulted veterinarian confirmed these observations and took blood samples of a subset of animals for a "new world camelid screening test". The most remarkable results of the blood analyses were the vitamin D levels markedly exceeding the reference values (all sampled animals). Furthermore a mare was hospitalized in the Clinic for Small Ruminants, University of Veterinary Medicine Hanover, Foundation, Hanover. The cachectic and azotaemic mare died due to cardiovascular failure shortly after arrival in the clinic. The plasma concentration of Ca was within the reference ranges, beside a hyperphosphataemia, and a hypermagnesaemia. During necropsy a hypoproteinaemic ascites and a low grade gastritis, a low to middle grade interstitial nephritis, a low to middle grade interstitial fibrosis of both kidneys and severe mineralized intratubular concrements were observed. Potential aetiologies discussed were an insufficient water intake, a hypercalcaemia or a vitamin D intoxication. For further diagnostic support the Institute of Animal Nutrition, TiHo, was consulted.

Material and methods: Feed samples were sent in (alfalfa hay and two complementary feed products for alpacas). First a visual appraisal/sensory evaluation of samples was done followed by a vit. D analysis. The Vitamin D_3-content was analysed by the LUFA SPEYER according to the method III 13.8.1 of VDLUFA (2012). In parallel, further information on feed amounts, feeding management and the labelled values on feed composition were gathered.

Results: The alfalfa hay was of green colour and contained no blossoms but a high proportion of stems. It smelled aromatic and was of bulky structure. There were no hints on the occurrence of toxic plants (e.g. trisetum flavescens), so it was not submitted to additional analyses. The green-brown and pelleted complementary feed 1 (CF1) had a low grade roasty smell whereas the dark green and pelleted complementary feed 2 (CF2) smelled low grade rancid. Both were dry in grip. The vitamin D_3 content of CF1 and CF2 were determined and compared with the labelled content. In CF1 the vit. D content was below the detection limit (< 1000 IU/kg diet) and no vitamin D-content/addition was declared on the product. The CF2 contained 29730 IU vit. D/kg diet which deviated beyond the analytical (VDLUFA 2014) and technical latitudes (Reg. (EC) 767/2009) from the declared content. For calculation of the daily vitamin D intake only the intake via CF2 (1.5 kg per animal per day according to the owner) was considered. The calculated vit. D intake was assumed to be not less than 44595 IU per animal per day.

Discussion and conclusion: Considering a body weight of 65 kg and a vit. D requirement of 30 IU/kg bw (NRC 2007) an intake of 1950 IU vit. D per animal per day would meet the requirement. According to the NRC (2007) small ruminants tolerate a diet which is offered for more than 60 days if the vit. D content does not exceed 2200 IU/kg DM. Considering a daily DM-intake of 3 % of the body weight including 1.5 kg of CF2 (no further vitamin D intake via further components/feeds) the daily ration contained not less than 22869 IU vit. D per kg DM. In conclusion, a marked vit. D oversupply due to overdosing of a complementary feed for alpacas (feeding recommendation: 1 g/kg body weight per day) seemed to be causative for the clinical and pathological findings in the alpacas. The labeling of the complementary feed for alpacas was correct: Due to the crude ash content of 17 % (declared value) it was not a mineral feed (crude ash content per definition > 40 %).

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100.

Prediction of starch and crude protein concentration of cereal and pea grains and their incubation residues from ruminal *in situ* studies using near-infrared spectroscopy

Schätzung des Stärke- und Rohproteingehalts von Getreide- und Erbsenkörnern sowie deren Rückständen aus in situ Experimenten mittels Nahinfrarotspektroskopie

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In situ incubations are widely used to investigate ruminal degradation characteristics of feedstuffs. Chemical analysis is time consuming and expensive. Availability of a fast and cheap method like near infrared spectroscopy (NIRS) to predict nutrient concentrations would be advantageous. Due to sample matrix effects and different methods of starch (ST) analysis in use, none of the available NIRS calibrations is suitable for application to incubation residues. Crude protein (CP) and ST concentrations of incubation residues vary to a much greater extent than in grains and are in most cases much lower. Therefore the objective was to establish NIRS calibrations to predict the ST and CP concentration of cereal and pea grains and their *in situ* incubation residues. We also compared the degradation characteristics calculated based on NIRS prediction with those obtained by chemical analyses.

Methods: Six hundred and twenty samples were chemically analysed for their ST (1) and CP concentration (2). The samples comprised rye, triticale, barley, wheat and corn grains (20 genotypes each), 15 durum wheat and 13 pea samples, and their residues after ruminal *in situ* incubations carried out in three cows over different time spans from 0 to 72 hours. NIR spectra of ground samples (0.25 mm) were recorded in duplicate using ring cups with inlays for small sample volumes (Spectrastar 2500X, Unity Scientific, Brookfield, CT). A subset of 150 samples was used for validation. With the remaining samples (ST: n=460, CP: n = 470), calibrations were computed by PLS-regression using 3 different wavelength segments: 680-2500 nm, 730-2450 nm and 1250-2450 nm (Ucalibrate, Version 3.0.0.23, Unity Scientific, Brookfield, CT). Mathematical treatment of spectra was varied: no derivation, 1st or 2nd derivation. Cross validation (CV) was performed in 5 groups and outliers were excluded from the calibration by a T-value limit of 2.5. The fit of the calibrations was evaluated by the RMSE of the calibration, the CV and the validation, the bias, slope and intercept of the validation.

Results: The calibrations based on the wavelength section 1250-2450 nm and using the 1st derivation showed the best results based on the validation characteristics. These calibrations were chosen for further calculations. The high R^2 and low bias as well as the validation slope and the intercept reflect the good performance of the calibrations. Generally the error of the estimated concentration was not affected by the concentration measured chemically.

Table Statistical parameters of calibrations for the prediction of starch (ST) and crude protein (CP) concentration (% of dry matter) using NIRS

	$RMSE_C$	R^2_C	$RMSE_{CV}$	R^2_{CV}	$RMSE_V$	R^2_V	$bias_V$	$slope_v$	$intercept_v$
ST	1.57	1.00	1.71	0.99	2.10	0.99	0.08	1.00	0.17
CP	0.35	1.00	0.39	0.99	0.46	0.99	0.03	1.00	0.07

RMSE root mean square error; C calibration; CV cross validation; V validation; R^2 coefficient of determination

Degradation characteristics (soluble fraction (a), potentially degradable fraction (b), degradation rate (c) and effective degradation (ED)) were computed for 84 samples (4 genotypes of 7 grain species, each incubated in 3 animals) based on chemical analysis and NIRS prediction of ST and CP concentration. Pairwise comparison showed no significant difference ($P > 0.05$) between ruminal degradation characteristics from data obtained by NIRS and chemical reference analysis.

Conclusion: NIRS can be used as an alternative to chemical analyses to determine the ST and CP concentration of cereal grains and their incubation residues from *in situ* degradation studies

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101.

Impact of sainfoin and birdsfoot trefoil on *in vitro* ruminal microbial protein synthesis

Einfluss von Esparsette und Hornklee auf die ruminale mikrobielle Proteinsynthese in vitro

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Rapid degradation of dietary protein may lead to high concentrations of ammonia in the ruminal fluid, the excess of which cannot be efficiently utilized by the microbes and thus causes energy and N loss (1). These losses lead to a reduced efficiency of microbial protein synthesis (2). The microbial protein together with the undegraded feed protein makes up the utilisable crude protein at the duodenum (uCP) which is, besides energy, the most limiting factor for animal performance. Bioactive compounds like condensed tannins (CT) may interfere in the ruminal degradation processes by building complexes with proteins. By this, the synchronisation of protein and carbohydrate degradation may be influenced and may change the efficiency of microbial protein synthesis.

The objective of the present study was to determine the impact of the two CT-containing legumes sainfoin and birdsfoot trefoil and their mixtures with red clover compared to lucerne and red clover alone on *in vitro* microbial protein synthesis.

Methods: Amounts of 12 g silage dry matter (DM) were incubated 6 times (n=6) using a rumen simulation technique with eight 1-L fermenters in a 39°C water bath. Test plants were lucerne (LU), red clover (RC), sainfoin (SF, 136 g CT/kg DM), birdsfoot trefoil (cultivars Polom (BTP) with 23 g CT/kg DM and Bull (BTB) with 35 g CT/kg DM) as well as 1:1 mixtures of each CT-containing legume with red clover. Incubations lasted for 10 days. The last 5 days were used for data and sample collection. Apparent degradability of crude protein (CP) and organic matter, ammonia (NH_3) concentration, purine content in the liquid- (LAM) and particle-associated microbes (PAM) as well as microbial protein synthesis by LAM and PAM were determined and uCP was calculated taking into account the N content of the silages and the NH_3 concentrations of blanks and incubation fluids. Data were analysed with the MIXED Procedure of SAS considering treatment as fixed effect and the run as random effect.

Results: The degradability of organic matter was lower (P3concentration was highest (P

Conclusion: The degree of ruminal protein degradation is lower with SF, likely by the binding of the protein with the CT, and the amount of synthesized microbial protein appears to be higher by SF and BTP in comparison to RC and LU. Thus, these results demonstrate several advantages of adding CT legumes strategically to ruminant diets. It has to be shown whether the CT-protein bonds are really dissolved in the lower gut.

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102.

Effects of replacing molassed dried sugar beet pulp for maize in concentrate-rich diets of dairy cattle on ruminal fermentation *in vitro*

Der Einsatz melassierter Trockenschnitzel als Ersatz für Mais in konzentratreichen Milchviehrationen und ihr Effekt auf das Pansenmilieu in vitro

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Introduction: Grain feeding increases dietary energy density but may also lead to ruminal dysfunction in cattle. Sugar beet pulp (BP) is a human inedible by-product of the sugar industry, and can be used as an energy rich feedstuff in ruminant nutrition. In contrast to grain, molassed BP contains no starch, but is rich in sugars and digestible fiber such as pectin. Compared to starch, the fermentation of pectin predominantly results in formation of volatile fatty acids and less of lactate (1). This event may reduce the acidic load of the rumen and enhance pH when pectin is used as a substrate. However, BP provides slightly less energy for rumen microbes than starch. The objective of this study was to evaluate the amount of maize grain that can be substituted by molassed dried BP regarding fermentation parameters using the rumen simulation technique (RUSITEC).

Methods: The experiment involved 6 different diets (0BP, 8BP, 16BP, 24BP, 32BP, 40BP) differing in the equivalent substitution of the amount of BP for maize such as 0% (no BP inclusion but 40% maize), 8%, 16%, 24%, 32%, and 40% BP inclusion (DM basis), respectively. The diets also contained (DM basis) 24% grass silage, 24% maize silage, 10% rapeseed meal, 0.9% limestone, 0.8% urea, and 0.3% mineral-vitamin premix. The CP content was maintained constant among the diets and averaged 15.6% (DM basis). The experiment was conducted in 3 different runs (n=6), each lasting for 10 days. Measurements and sampling were only performed during the last five days of the experiment. The pH was measured daily before, and 2, 4, 6 and 8 hours after feeding using an electrode. The RedOx-potential and fermentation gases were measured daily before feeding. Samples for determination of ammonia concentration were taken daily before feeding and frozen at -20°C and for subsequent analysis. Statistical analysis was performed using the MIXED procedure of SAS.

Results: The substitution of maize for BP linearly lowered the total starch content from 40% in the 0BP-diet to 10.5% in 40BP-diet, but increased the sugar content from 1.8% in the 0BP-diet to 6.1% in 40BP-diet. Feeding of BP significantly affected ruminal pH in a way that mean pH during the first 8 hours after feeding increased ($p<0.01$) from 6.38 for 0BP-diet to 6.51 for the 40BP diet. The minimum and maximum pH values followed this increasing trend as well. The ammonia-N concentration increased ($p<0.01$) from 12.99 mmol/l to 14.49 mmol/l with increasing the amounts of BP in the diet. Also, the amount of methane produced daily was higher ($p<0.01$) with increasing the substitution rate of BP in the diet. The Redox-potential averaged -237,1mV among treatments from day 1 to 5. From day 6 to 10 the Redox-potential decreased ($p<0.01$) with increasing the inclusion rate of BP in the diet from -181,4mV to -221,1mV for 0BP to 40BP, respectively.

Conclusion: The data suggest that the use of molassed BP as a major energy component in the diet enhances ruminal pH. However, because of the increased ammonia concentration and greater methane formation, the feeding of large amounts of BP may lower energy availability and therefore negatively impact fermentation output and possibly the microbial protein synthesis in the rumen. Further analyses are needed to evaluate the effect of BP feeding on the rumen fermentation.

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103.

Measuring the linear buffering capacity in fibre rich feed sources

Bestimmung der linearen Pufferkapazität von faserreichen Futtermitteln

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Introduction: There is a lack of information about intrinsic physicochemical properties of feeds passing the gastrointestinal tract (GIT). The buffering capacity (BC) is the ability of the feed to oppose a pH change in the GIT. Depending on its BC, feed components like fibre exchange cations with H^+ in the lumen of the GIT. The knowledge of this physicochemical property of feeds is important to balance a diet for e.g. piglets with limited capacity of HCl production in the stomach or to influence nutrient absorption in the small intestine. The objective was to determine the linearized form of the BC (the linear buffering capacity rate, LBR), and to rank fibre sources for their linear BC.

Methods: To measure the buffering capacity, 1g sample (1mm sieve) is soaked in 100ml distilled water and stirred for 30min. The initial pH was determined and the pH of these suspensions were raised to 8.0 with NaOH and then titrated with HCl until pH 2.0 was achieved. The TA (titratable acidity) was determined as the amount of acid needed to reduce the pH from 8.0 to 2.0, expressed in mEq/g dry matter of the sample. The pH data were transformed by the function y= exp (1/pH) and the linear buffering rate (LBR) was calculated as the inverse of the slope of the linear regression between y and the cumulated amount of acid added (1). The data of LBC were statistically analysed by the GLM procedure of SAS (ANOVA) as completely randomized design. Fibre rich feeds analysed are: soybean hulls, spelt hulls, beet pulp, apple pomace, sunflower hulls and two types of lignocellulose (A and B).

Results: Fibre (total dietary fibre, crude fibre) and crude protein contents are presented in Table 1. To achieve the pH decrease from 8 to 2 for the samples 2.12 mEq H^+/g DM were necessary on average, achieving highest values for soybean hulls B (2.89 mEq H^+/g DM) to lowest values for lignocellulose FC (1.34 mEq H^+/g DM). Titrated acid and pH shift of the analysed samples are summarized in figure 1. The calculated LBR are highest for soybean hulls and beet pulp. The lowest values are for spelt hulls, lignocellulose A and lignocellulose B (Table 1). Correspondingly, the amount of acid needed to decrease the pH of feeds linearly increase with LBR.

Table 1: Linear buffering capacity rate (LBR) of fibre rich feeds, as well as TDF, CF and CP contents (in % DM).

	LBR	r^2	Total dietary Fibre (TDF)	TDF soluble	TDF insoluble	CF	CP
lignocellulose A	2.44^d	0.86	95.30	1.13	94.17	60.45	0.66
lignocellulose B	2.47^d	0.85	94.51	1.26	93.26	53.13	1.11
spelt hulls	2.69^d	0.85	87.25	0.61	86.63	40.09	2.21
apple pomace	3.69^c	0.96	67.63	14.62	53.01	24.15	6.91
sunflower hulls	4.57^b	0.92	n.a.	n.a.	n.a.	54.46	4.26
beet pulp	5.08^a	0.97	63.69	16.32	47.37	16.29	9.17
soybean hulls	5.18^a	0.96	65.44	6.97	58.47	29.29	17.85

a,b Means without a common superscript differ ($P<0.05$)

Conclusion

The study demonstrated that the measurement of linear buffering capacity is useful to differentiate different fibre rich feeds for their physicochemical properties. These may influence the choice of fibre rich feeds in piglet nutrition.

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104.

Different feeding intensities during the first four weeks of rearing in dairy calves: Part 2: Effects on the metabolic and endocrine status around the first lactation

Einfluss der Fütterungsintensität innerhalb der ersten 4 Lebenswochen auf den metabolischen und endokrinologischen Status in der 1. Laktation

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Intensified feeding strategies over the first weeks after birth to feed the calves with higher amounts of nutrients from milk replacer or whole milk may alter the phenotypic expression for milk yield. Some recent studies and meta-analyses comparing the effects of controlled nutrient intakes or *ad libitum* (*al*) feeding of calves from birth up to 56 days of life have found that an increasing nutrient intake before 56 days of life from milk resulted in increased milk yield from 450 to 1,300 kg during the first lactation compared with the milk yield of restricted-fed calves during the same period (1; 2). Much less literature is available about the metabolic and endocrine status from the weaning period of calves over the first lactation when calves were fed with divergent feeding strategies (restrictive or *al*). For this reason we conducted a feeding trial with different feeding intensities of *al* feeding of whole milk (WM) or milk replacer (MR) and of restrictive (r) feeding of MR during the first 4 weeks of life to test for potential effects on the metabolic and endocrine status in the first lactation (data about the first 110 days of life were reported elsewhere (3)).

Material and Methods: Female German Holstein calves (n = 28) were allocated directly after birth to 3 groups: MRr (n = 10; 6 L MR/calf/day), MRal (n = 9) and WMal (n = 9). Colostrum was fed for the first 3 d post natum (p.n.) whereby group MRal and WMal had al and group MRr had restricted (6 L/d) access. Thereafter all calves received the respective feeding during the first 28 d of life. Thereafter all calves were fed according to the MRr regimen, and were then weaned, further raised, bred and kept as common practice. Blood samples from the calves were taken regularly in the 108 days p.n. and from the young heifers monthly starting 3 months before expected calving and weekly over the first 10 weeks of lactation. The blood samples were analysed for NEFA, glucose, BHBA, insulin and adiponectin. Statistical analysis was performed with SPSS 22 using a mixed model.

Results: In the heifers fed at divergent feeding intensity during the first month of age, no sustained effects were detectable when reaching their first lactation in either blood variable assessed. The animals showed the characteristic peripartal changes of the concentrations but the patterns were not different between the groups.

Conclusion: The feeding regimen applied during the first month of age affected the circulating concentrations of glucose, insulin and NEFA exclusively during the time of differential feeding (3), but not thereafter, i.e neither until the end of the first observation period (until d 110 of age; 3) nor around the first lactation. These results point towards a flexible compensation of nutrition during early life rather than to a sustained programming of metabolism.

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105.

Comparison of feed intake patterns of sport ponies *vs* warmblood type horses following iso-energetic intake of three differently confected commercial concentrate feeds

Vergleich von Futteraufnahmecharakteristika von Sportponys vs. Warmblutpferden bei isoenergetischer Aufnahme von drei unterschiedlich konfektionierten, handelsüblichen Konzentraten

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Chewing patterns vary between feedstuffs and treatments with resultant different saliva production and probable consequences regarding gut health and metabolic properties. Previously it has been shown that ponies of older genotypes need more time to ingest equal amounts of concentrates and roughages than horses (1). In another study, where unfortunately no direct comparison between breeds was made modern-type sport ponies seamed to consume concentrate feed as rapid as known from warmbloods (2). From that we hypothesized that modern-type sport ponies ingest concentrate feed at a similar rate as horses. Aim of the study was to compare feed intake patterns of modern-type sport ponies and warmbloods after the ingestion of different types of concentrate feeds.

Methods: Four sport ponies (age 8.0 ± 3.1 years; body weight [bwt] 370 ± 12.9 kg; body condition score [BCS] 5.2 ± 0.21/9) and six warmblood type horses (age 8.5 ± 3.1 years; bwt 516 ± 40.9 kg; BCS 5.04 ± 0.12/9) were individually housed in box stalls with straw as bedding and received metabolizable energy (ME) according to maintenance (0.52 MJ ME/kg $bwt^{0.75}$ d^{-1}; 3). Both breeds were allocated per random onto 3 groups and got once per day either semi-crushed oat grains (OG; 1 g starch/kg bwt meal $^{-1}$; per kg dry matter [dm]: 393 g starch, 160 g acid detergent fibre [ADF], 31 g acid detergent lignin [ADL], 12.5 MJ ME) or iso-energetic quantities of a pelleted fiber-rich mixed feed (PF; per kg dm: 308 g starch, 281 g ADF, 55 g ADL, 9.3 MJ ME) or a muesli feed (MF; per kg dm: 135 g starch, 189 g ADF, 37 g ADL, 10.6 MJ ME) according to a cross over design with period length of 8 days. Hay covered the remaining energy need. On d8, feed intake patterns were measured by a modified halter (2). SAS® was used for statistical analysis, two-way ANOVA was performed.

Results: Ponies and warmbloods kept their bwt during the study ($P > 0.05$). During adaption to PF, increasing saliva production was observed. In warmbloods, PF tended to be ingested faster than MF followed by OG (min/kg dm: 10.3 ± 1.85, 13.0 ± 1.30, 14.4 ± 2.01; $P > 0.05$). In ponies, the counterparts were PF 13.5 ± 2.47, MF 13.6 ± 2.01 and OG 15.4 ± 1.11 min/kg dm ($P > 0.05$). Ponies *vs* warmbloods needed more time to ingest PF ($P < 0.05$). In warmbloods, the chewing frequency (in chewings sec^{-1}) was not affected by the type of concentrate (PF 1.45 ± 0.176 MF 1.40 ± 0.157, OG 1.41 ± 0.112), but in ponies OG was chewed with a particularly low frequency (PF and MF, 1.43 ± 0.11 *vs* OG 1.28 ± 0.093; $P < 0.05$). There were no significant differences between concentrates and breeds regarding dm intake within a given time or the number of chewings expended per kg of dm.

Conclusion: The results suggest that concentrate intake of warmbloods and modern type sport ponies follows similar patterns. Breed differences were limited to PF and although significant in absolute terms of minor importance. From this, it can be speculated that sport ponies ingest equal amounts of concentrate feed similarly rapid as larger sized horses. Concerning allometric effects, this might increase the susceptibility for oesophageal obstruction in ponies. Furthermore, a more rapid ingestion of starchy concentrates as previously thought should cause altered glycaemic and insulinaemic responses with probable metabolic consequences for individuals with disturbed insulin sensitivity.

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106.

Chewing frequency and moved distances of warmblood-type horses all-day grazing on an extensive pasture

Kaufrequenz und zurückgelegte Wegstecken von Warmblutpferden auf extensiver Weide

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Free ranging horses on pasture spent up to 17 h/d grazing while in stabled horses with restricted feed supply this period might be limited to less than 4 h/d (1). Feed intake promotes chewing which in turn is of enormous importance for the horses' gut health. Chewing patterns, however, are not only dependent from the type of the feed but also its treatment and further diverse concomitant factors such as pre-feeding characteristics and housing conditions. To our knowledge, no results on chewing patterns from all-day grazing horses exist, but for horses with short term grazing or intake of cut meadow grass in the stable limited data are available where the reported chewing frequencies [CF] are close to those for meadow hay (2). Ponies, however, seem to be able to elevate the speed at which they eat if the grazing time is reduced following periods with extended grazing (3). We hypothesized that in all-day grazing horses the particular farming condition with notably permanent availability of food diminish the frequency with which they chew. The aim of the study was to measure CF of horses subjected to all-day grazing on an extensive pasture and characterize the distances they move.

Methods: Six warmblood mares (age 8.5 ± 3.1 years; body weight [bwt] 516 ± 40.9 kg; BCS 5.3 ± 0.33/9) adapted to all-day grazing were kept for 5 days (late spring) on rotational pasture (altogether 3 cycles) for 24 h/d. Prior to and during the study the pasture was subjected to botanical scoring. Every day feed intake patterns were measured by a modified halter for 13 h/d (7:00 a.m. - 8:00 p.m.). Additionally the modified halter was prepared with a GPS G5 sensor (POLAR®, Büttelborn, Germany) to measure the distances the horses moved at different velocities. The meadow grass was analysed for proximate nutrients. The intake of dry matter (dm) was estimated (2).

Results: Major gramineous components on the pasture were in descending order oatgrass, brome grass and cocksfoot, and further nettles as herbage. Perennial ryegrass, meadow fescue as well as red fescue were below 5% each. Feed analysis revealed the following (per kg dm): dm, 203 ± 9.05 g; crude protein, 156 ± 35.1 g; crude fiber, 303 ± 15.2 g; crude lipids, 22 ± 3.7 g; crude ash, 85 ± 8.9 g; 6.9 ± 0.44 MJ metabolizable energy. The horses kept their bwt during the study ($P > 0.05$). The median CF was < 1 chewing cycle [CC]/sec in all horses, breaks in feed intake and chewing within the observed time ranged from 5 - 35 min/h, except for a maximum of 65 min/d in three horses at 1 day only. Although the sub-pastures' sizes were small (12 livestock units/hectare), horses covered an averaged distance of 1 - 2 km/h. The individual variation of dm intake during every 13 h measuring period/d was estimated to be 0.6 - 0.8 kg/h (≙ 1.4 - 2.1 % bwt) (2).

Conclusion: Result suggests that in all-day grazing horses the CF is slowed down if compared with other feedstuffs (concentrates, hay, straw) or meadow grass consumed during short time grazing or as cut material in the stable. Despite the calculated quantities of dm intake do not seem to be unrealistic if compared to literature, potentially there is an overestimation, because Dill (2008) detected a CF > 1CC/sec and horses got a time limit on pasture. The horses' motional activity was remarkably high, although only a restricted area was available. Thus, the positive physiologic impact of full-time pasturing seems to be evident. In practice, further conditions concerning the pasture need to be considered. Upcoming studies should aim to measure the horses' CF during a 24 h-period on pasture.

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107.

Expander processing of maize increases digestibility of nutrients and hence alters the lysine to energy ratio in diets for fattening pigs

Expanderbehandlung von Mais und dessen Einfluss auf die Nährstoffverdaulichkeit und das Lysin:Energieverhältnis beim Mastschwein

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Expander processing, known as high temperature short time (HTST) technology, is widely used in the feed industry and designed to alter the physicochemical properties of feed components. Especially the high specific energy inputs (kWh/t) used in single components processing like maize before mixing leads to a pronounced starch gelatinization and disruption of cell walls. An increase of about 10% of the digestible energy (DE) is expected (1) and the HTST causes a deviation in the lysine to energy ratio (Lys:E ratio). However, the intensity of amino acids (AA) damage on the further deviation in Lys:E ratio and its influence on animal performance is unclear. The aim of the present study was to determine the effect of an intensive expander treatment of maize on apparent total tract digestibility (ATTD) and carcass quality.

Methods: After grinding (hammer mill, 2mm sieve, belonging to the same batch) maize remains HTST unprocessed (control diet (C)). HTST processed, replacing by 100% HTST unprocessed maize, were used for treatments SC, LC and LC+AA. In these treatments, maize was short- (60 s, SC) and long-term (1080 s, LC) conditioned (80°C) and subsequently intensively expanded (~45 kWh/t, OEK 15, A. Kahl, Germany). Processed or unprocessed maize was mixed with further components (soybean meal, wheat bran, premix, TiO_2) at the same proportion (grower: 66%; finisher: 65%) to achieve requirements (2). In treatment LC+AA, AA content was increased by 10% to maintain the ideal protein. The experiment used 60 crossbreed barrows (30.7±0.3 kg), which were assigned to one of four treatments. Mash feed and water were provided *ad libitum*. At slaughter, faeces samples were collected from rectum to evaluate ATTD. Additionally, fattening and slaughter parameters were recorded. Data were analysed using ANOVA as randomized block design, with Tukey-Kramer test for LS-mean separation ($P<0.05$).

Results: Expanded maize improved DM and EE ATTD and DE content (Table 1). Within expander treatments, SC maize had higher ATTD for DM and DE content, followed by LC and unprocessed maize (C). Considering the maize proportion in diets, the amount of DE/kg DM of maize reached 8.4%, 5.1% and 4.1% in SC, LC and LC+AA respectively, compared to the control. Supplemented AA did not affect ATTD-parameters, but reduced feed intake and feed conversion rate (FCR). Expanded maize declined loin depth and lean percentage, which was reversed in LC+AA treatment, while no influence of the average daily gain (ADG) was observed.

Table 1: Effect of expander treatment of maize on feed intake, FCR, ADG and the ATTD of nutrients and their effect on selected carcass characteristics

Item	C	SC	LC	LC+AA	SEM	P-Value
Reactive lysine, g/kg DM	1.76[1]	1.69	1.65	as LC	-	-
Feed intake[2], kg DM/d	2.4^{ab}	2.4^{a}	2.4^{ab}	2.2^{b}	0.03	<0.05
FCR[2], kg/kg	2.6^{ab}	2.7^{b}	2.6^{ab}	2.4^{a}	1.01	<0.05
ADG[2], g/d	938	891	913	899	8.33	>0.10
ATTD of DM, %	82.58^{c}	87.13^{a}	84.99^{b}	84.64^{b}	0.34	<0.05
ATTD of EE, %	52.44^{b}	73.35^{a}	71.47^{a}	71.38^{a}	1.02	<0.05
MJ DE/kg DM	16.10^{c}	16.98^{a}	16.65^{ab}	16.54^{b}	0.63	<0.05
depth back fat (mm)	20.7	21.8	20.6	19.9	0.18	>0.10
loin depth (mm)	74.5^{ab}	73.2^{b}	74.2^{ab}	77.4^{a}	0.51	<0.05
lean, %	60.1$^{(a)}$	58.9$^{(b)}$	59.8$^{(a)}$	60.8$^{(a)}$	0.24	<0.10

[1] after drying

[2] average of the whole period (31-116 kg LW)

a,b Means within a row without a common superscript differ ($P<0.05$)

(a,b) Means within a row without a common superscript differ ($P<0.10$)

Conclusion: There is a potential to improve the nutrient digestibility of individually expander treated feeds for pig diets. However, the Lys:energy ratio should be considered in diet formulation, to avoid higher fat proportion in the carcass.

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108.

Protein hydrolysates from animal by-products as sustainable protein source. I: Effect of thermal hydrolysis and origin of basic material on protein quality

Proteinhydrolysate aus Schlachtnebenprodukten als zukunftsfähige Proteinquelle. I: Einfluss der Hydrolysebedingungen und des Ausgangsmaterials auf die Proteinqualität

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Recently, the European parliament is debating about legalizing the application of processed animal proteins, e.g. novel protein hydrolysates (PH) from animal by-products, as a sustainable alternative for conventional protein sources in livestock feed. However, limited information is available regarding the protein quality and protein utilization of these PH. The aim of the current study was to evaluate the effects of hydrolysis and basic material on protein quality of lab scale processed PH.

Methods: A N-balance study (5d adaption + 5d collecting period) was conducted using five isonitrogenous and isoenergetic semi-synthetic diets formulated to contain either 9.25% casein as reference protein (Control) or adequate amounts of PH (8.85-8.90%) originating either from rendered pork (PH I/II) or poultry (PH III/IV) material (Category 3). Feed amino acids were not supplemented. PH (lab scale production; ANiMOX GmbH, Berlin) of respective diets were specified as follows: PH I/III = enzymatic pre-hydrolysis (60°C, 6h) followed by thermal hydrolysis (autoclaving at 130°C, 2h), lyophilization of supernatant, 96.0-96.1% DM, 90.4-91.2% CP, 3.9-4.1% CA; PH II/IV = enzymatic pre-hydrolysis (60°C, 6h) followed by autoclaving of solid residues (130°C, 2h), subsequent fusion and lyophilization of both supernatants, 95.7-96.0% DM, 90.2-90.6% CP, 4.2-4.9% CA. Diets were fed to 35 juvenile male Wistar rats (n=7/diet; daily feed intake restricted to 10g/rat) to obtain N-balance data. True N-digestibility (tND), biological value (BV) and standardized net protein utilization (PNU_{std}) were calculated according to (1), except applying a standardized N-intake (NI) of 1174mg/$BW_{kg}^{0.67}$ as observed on average. Statistical analysis (one-way ANOVA, Tukey-test) was conducted by R-software (version 3.0.2).

Results: Results of the experiment are presented in the table below. All diets were well accepted by the rat. Control diet and PH II/IV yielded higher tND than PH I/III, indicating negative effects of total autoclaving (i.e. extended heat treatment). BV between diets was nearly similar with PH III showing highest values. In this context, higher tND was mainly accompanied by lower BV. Considering PNU_{std}, PH I-IV were inferior to control diet despite significant differences were not generally achieved due to high variances (PH III/IV). PNU_{std} between PH I-IV showed similar data.

Diet	Control	PH I (pork)	PH II (pork)*	PH III (poultry)	PH IV (poultry)*
tND [%]	$92.9^{a} \pm 1.5$	$77.2^{b} \pm 10.0$	$86.8^{ab} \pm 3.3$	$77.5^{bc} \pm 9.5$	$4^{ac} \pm 3.3$
BV [%]	$65.4^{ab} \pm 5.0$	$58.9^{ab} \pm 6.3$	$55.7^{a} \pm 6.2$	$69.3^{b} \pm 13.0$	$59.0^{ab} \pm 9.2$
PNU_{std} [%]	$60.0^{a} \pm 4.5$	$45.8^{b} \pm 6.3$	$48.3^{b} \pm 5.2$	$53.4^{ab} \pm 9.6$	$51.4^{ab} \pm 8.0$

Means (± SD); tND = true N-digestibility; BV = biological value; PNU_{std} = standardized net protein utilization (standardized N-intake = 1174mg/$BW_{kg}^{0.67}$); * thermal hydrolysis limited to solid residues from enzymatic pre-hydrolysis; different superscript letters reveal significant differences between diets (p

Conclusion: Despite exhibiting lower protein quality compared to casein as control, the evaluated lab scale processed protein hydrolysates originating either from rendered pork or poultry by-products suggested a potential to act as sustainable protein source in livestock feed. In this context, it is recommended to prefer further on the basic material from poultry by-products. Additionally, negative effects of total autoclaving during PH processing on protein digestibility should be considered. Further research is needed to optimize treatment conditions for minimized damage of the protein fraction of processed animal proteins.

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109.

Protein hydrolysates from animal by-products as sustainable protein source. II: Protein quality of industrial scale processed products

Proteinhydrolysate aus Schlachtnebenprodukten als zukunftsfähige Proteinquelle II: Proteinqualität industriell hergestellter Produkte

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An increasing global demand for animal products in human nutrition accompanied by limited availability of conventional protein sources will cause severe challenges regarding protein supply in livestock feeding. Novel protein hydrolysates (PH) from animal by-products could offer a sustainable alternative to the feed industry. The aim of the current study was to evaluate the effect of industrial scale production on protein quality of the mentioned PH for further application in livestock feeding using the laboratory rat as a model.

Methods: A N-balance study (5d adaption + 5d collecting period) was conducted using five isonitrogenous and isoenergetic semi-synthetic diets formulated to contain either 9.25% casein as reference protein (Control) or adequate amounts of PH (8.85-9.20%) originating either from rendered pork (PH VII/VIII) or poultry (PH V/VI) material (Category 3). Feed amino acids were not supplemented. PH (industrial scale production; ANiMOX GmbH, Berlin) in diets were specified as follows: PH V/VI = autoclaving at 160°C, 30min (V) vs. 60min (VI), lyophilized, 96.2-96.6% DM, 90.5-90.8% CP, 0.3-0.4% CL, 4.5-4.7% CA; PH VII/VIII = autoclaving at 160°C, 80min, with (VIII) or without (VII) 40% bristle added, spray-dried, 96.1-97.2% DM, 87.5-89.8% CP, 1.1-2.2% CL, 3.1-3.4% CA. Diets were fed to 35 juvenile male Wistar rats (n=7/diet; daily feed intake restricted to 12g/rat) to obtain N-balance data. True N-digestibility (tND), biological value (BV) and standardized net protein utilization (PNU_{std}) were calculated according to (1), except applying a standardized N-intake (NI) of 1220mg/$BW_{kg}^{0.67}$. Statistical analysis (one-way ANOVA, Tukey-test) run with R-software (version 3.0.2).

Results: Results of the experiment are presented in the table. All diets were well accepted by the rat. Control diet outperformed all PH diets regarding tND, BV and PNU_{std}. PH V/VI showed higher tND than PH VII/VIII indicating negative effects of processing conditions (e.g. autoclaving period) and/or origin (pork vs. poultry) of respective PH. No differences could be observed between PH V-VIII regarding BV. Considering PNU_{std} higher values for PH V/VI than VII/VIII were observed, likely caused by differences in tND. Both heat treatment during PH processing (PH V vs. VI) and inclusion of bristle (PH VII vs. VIII) did not provide significant effects on tND, BV and PNU_{std}, respectively.

Diet	Control	PH V (poultry)[1]	PH VI (poultry)[2]	PH VII (pork)[3]	PH VIII (pork)[4]
tND [%]	$93.0^{a} \pm 1.2$	$68.4^{b} \pm 6.2$	$69.2^{b} \pm 5.3$	$60.2^{c} \pm 3.6$	$54.5^{c} \pm 5.7$
BV [%]	$65.7^{a} \pm 4.1$	$45.6^{b} \pm 4.1$	$49.4^{b} \pm 6.5$	$48.6^{b} \pm 4.4$	$52.1^{b} \pm 9.0$
PNU_{std} [%]	$59.2^{a} \pm 3.3$	$31.1^{bc} \pm 3.1$	$34.1^{b} \pm 3.8$	$29.4^{bc} \pm 2.9$	$28.2^{c} \pm 2.5$

Means (± SD); tND = true N-digestibility; BV = biological value; PNU_{std} = standardized net protein utilization (standardized daily N-intake = 1220mg/$BW_{kg}^{0.67}$); [1,2] autoclaving at 60°C, 30min ([1]) or 60min ([2]); [3,4] autoclaving at 80°C, no ([3]) or 40% ([4]) bristle added; different superscript letters reveal significant differences between diets ($p<0.05$).

Conclusion: Industrial scale processed protein hydrolysates originating either from rendered pork or poultry by-products exhibited only low protein quality data as compared to casein control diet and therefore, in contrast to products previously under study (2), demonstrated less potential as adequate sustainable protein source and ingredient for animal feeds. Further research is needed to identify the background of inadequate processing conditions. Additionally, the origin of basic material for production of protein hydrolysates needs more consideration.

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110.

Influence of soaking and germination before ensiling on the buffering capacity and content of water soluble carbohydrates in Jack bean and cowpea grains

Einfluss von Quellen und Keimen vor der Silierung auf die Pufferkapazität und den Gehalt an wasserlöslichen Kohlenhydraten in Körnern von Riesen- und Kuhbohne

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Due to adverse weather conditions in the tropics, ensilage is a considerable alternative for cost-intensive drying processes in order to preserve legume grains. However, a low ensilability of these feedstuffs is indicated by a high buffering capacity (BC) and a low content of water soluble carbohydrates (WSC). The aim of the study was to evaluate whether soaking and germination of Jack bean (*Canavalia ensiformis*) and cowpea (*Vigna unguiculata)* grains before ensiling might contribute to overcome these drawbacks.

Methods: Ripe and air-dry Jack bean (JBN) and cowpea (CWP) grains (100 g each, n = 4) were weighed in 600 ml beakers and soaked in deionized water. Three soaking times (ST; 18, 24, 30 h) and four ratios of grain:water (1:2, 1:3, 1:4, 1:5 [w:v]) were incubated at 30 °C to simulate tropical temperatures. For germination, beans were disinfected (1% NaClO) and thoroughly rinsed afterwards before being soaked (9 h) in deionized water at a ratio of 1:3 (w:v). Sterile dishes were lined with filter paper spread with cotton wool and sprayed with deionized water. JBN and CWP samples (n = 3) were distributed on the dishes and collected at 12, 24, 48 and 96 h and 12, 24, 48 and 72 h, respectively. The trial was conducted under a light and darkness regime in a temperature regulated chamber (25 ºC). Dry matter (DM), crude protein (CP), crude ash (CA), major elements, BC (1) and WSC were determined for every collected sample. Furthermore, the effect of germination was tested for a possible reduction of canavanine (2) in JBN and trypsin inhibitory activity (TIA, 3) in JBN and CWP. Means were compared using a General Linear Model (SPSS, version 19). The level of significance was preset at $P < 0.05$.

Results: Increasing ST and lower grain:water ratio promoted the reduction of BC ($P < 0.001$). After 30 h, BC was reduced from 8.5 and 6.1 to 5.8 and 3.3 g lactic acid·100 g^{-1} DM in JBN and CWP, respectively. Germination increased WSC ($P < 0.05$) in JBN (by 25%) and CWP (by 321%) after 96 and 72 h, respectively. Changes in CP were negligible, but CA decreased ($P < 0.001$) as ST and grain:water ratio rose with lower contents of calcium, phosphorus, magnesium and potassium ($P < 0.05$). Germinated CWP under light regime showed a lower ($P < 0.001$) TIA (60.44 mg TI·g^{-1} DM) than those germinated in darkness (66.85 mg TI·g^{-1} DM), but no effect was observed for JBN ($P > 0.05$). After 12 h of germination, the lowest TIA ($P < 0.05$) was detected with a reduction of 59% and 72% for JBN and CWP, respectively. Canavanine was unaffected ($P > 0.05$) by the illumination regime. After 12 h of germination only a 14% reduction was achieved ($P < 0.01$).

Conclusions: Soaking and germination reduced BC and increased WSC in legume grains of JBN and CWP and therefore likely will improve ensilability. Whether combined soaking and germination enhances BC and WSC at the same time should be investigated in further studies. The drastic reduction of canavanine and TIA after 12 h suggests extra beneficial effects, although it is not clear if these changes would have an effect on BC and WSC and *vice versa*. Although, neither soaking nor germination contributed to spoilage of the beans, an upscaling of the experiment under tropical conditions is recommended.

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111.

Influence of an increasing content of field pea (*Pisum sativum* L.) and or field beans (*Vicia faba* L.) in the feed on the growth of broiler chickens

Einfluss einer steigenden Konzentration an Felderbsen und oder Ackerbohnen im Futter auf das Wachstum von Mastbroilern

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Soybean meal is the most common protein source in diets of broiler chickens. The majority of this soybean meal is imported from non-European countries and often derived from genetically modified varieties. Therefore the interest has increased to research on the use of alternative local protein sources derived from non-genetically modified varieties, such as grain legumes. Therefore the following study was carried out to investigate the influence of increasing proportions of field peas (*Pisum sativum* L.), field beans (*Vicia faba* L.) and a combination of both sources in broiler diets on the growth performance.

Methods:

640 one-day old male cockerels (ROSS) were randomly distributed in 8 treatment groups over a study period of 31 days. Feed and water were provided for ad libitum consumption. Live weight was recorded for each broiler individually whereas feed was weighed back weekly on a pen-basis. Soybean meal (32%) was gradually replaced by peas (15/30%) or beans (5/10/20%) or totally replaced by peas (51%) and peas+beans (30%, 23%) in the diets. The field pea variety "James" contained 236 g/kg crude protein. The field bean variety "Tiffany" is a bean with a low content of the antinutritional factors vicine and convicine and 296 g/kg crude protein. All diets contained a balanced concentration of essential amino acids (Lys, Met, Thr, Trp). But only a protein content of 18/19% could be carried out in diets with 51% peas and 30% peas+23% beans since no further protein sources was used. Data were analyzed via ANOVA (SAS) and the Student-Newman-Keuls-test.

Results and conclusion:

The results of this study indicate that an inclusion rate up to a level of 30% field peas or 20% field beans in broilers' diet was without negative effects on feed intake and on growth performance (Table 1). A complete exchange of soybean meal by field peas (51%) or by field peas (30%) plus field beans (23%) reduced significantly the daily feed intake and daily weight gain and increased the feed to gain ratio.

Table 1 Feed intake, weight gain, final body weight and feed to gain ratio of broilers (mean)

Group	Feed stuff (%)			Feed intake, g/d	Weight gain, g/d	Final body weight, g	Feed to gain ratio, g/g
	Soya	Pea	Bean				
1	32	-	-	82.1 a	60.7 c	1925 c	1.352 b
2	26	15	-	84.3 a	62.7 bc	1985 bc	1.346 b
3	20	30	-	85.2 a	64.9 ab	2054 ab	1.313 c
4	-	51	-	59.9 b	40.9 d	1310 d	1.464 a
5	30	-	5	84.1 a	64.6 ab	2044 ab	1.302 cd
6	27	-	10	84.5 a	66.1 a	2092 a	1.278 d
7	22	-	20	84.5 a	65.0 ab	2058 ab	1.299 cd
8	-	30	23	59.8 b	40.2 d	1290 d	1.487 a

a,b,c,d Means with different letters differ significantly within columns ($p<0.05$)

Conclusion: This study demonstrated that partly replacing the most common protein source soybean meal by field peas up to a proportion of 30% or field beans up to a proportion of 20% had no adverse effect on feed intake and growth performance of broiler chickens.

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112.

Effect of technical feed treatment on performance and the digestive tract of broilers

Einfluss verschiedener Futterbehandlungen auf die Leistung und den Verdauungstrakt von Broilern

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Beside the positive effects of technical feed treatment such as improving feed quality by increasing of storage suitability, avoiding of separation or increasing performance of animals, also negative impacts especially on health need to be considered. The present study compares different technical feed treatments of a common diet with regard to effects on performance and the digestive tract of broilers.

Methods: A total of 624 broilers (Ross 308) were fed a common fattening diet (maize/soybean meal/wheat) differing in particle size (Cg - coarse, 60 % >1000 µm vs. Fg - fine, 60 % < 1000 µm) and in hydro-thermal treatment (HTT) (M - meal, without; P - pelleted; ExP - expanded and re-pelleted). Animals were housed in 48 floor pens containing 13 animals per pen in a climate controlled building on chopped straw. Feed and water were available *ad libitum*. Body weight (BW) and feed intake were recorded weekly. Two animals per pen were slaughtered at an age of 35 days. Selected digestive organs were morphologically characterized with regard to weight and dimensions. Dilatation of *Isthmus gastrici* was assessed by a scoring system (ISC; 0 = physiological, no obvious dilatation - 3 = high degree of dilatation).

Results: Caused by higher daily feed intake (DFI) and lower feed wastage HTT feed increased significantly average daily gain (ADG) and decreased the feed to gain ratio (FGR) of animals compared with their meal fed counterparts ($p < 0.05$). Broilers fed meal feed had heavier proventriculus and gizzards related to BW compared to animals of other feeding groups. HTT feeding increased significantly the length of proventiculus compared to meal fed broilers except for FgExP. The proventriculus was wider when fed CgP, FgP or CgExP compared with CgM. ISC was significantly higher when fed CgP and CgExP compared to CgM and FgExP. The width of proventriculus and the ISC correlated directly with DFI. The weight and the length of small intestine were significantly influenced by HTT and particle size. Small intestines of animals fed CgM were significantly heavier related to BW than intestines of animals of other feeding groups. Small intestine of broilers fed coarse feed was significantly shorter than intestine of animals fed finely ground feed. Animals fed FgM had significantly shorter intestines than broilers fed FgP. A different development of the *tunica muscularis*could be the reason for higher intestinal weight despite the decreased length of intestine in case of animals fed meal feed.

Conclusion: A high DFI caused by pelleted feed increased the prevalence of proventriculus-megalia and dilatation of *Isthmus gastrici*. The study emphasised the conflict between economic interests and animal health.

	Coarse grinding			Fine grinding			p - Value		
	M	P	ExP	M	P	ExP	Particle size (PS)	Hydrothermal treatment (HTT)	PS x HTT
Performance (n = 104)									
DFI [g]	61^{c}	95^{a}	83^{a}	63^{c}	97^{a}	76^{b}	0.621	< 0.001	0.001
ADG [g]	39^{e}	65^{b}	58^{c}	41^{e}	69^{a}	54^{d}	0.254	< 0.001	<0.001
FGR [g/g]	1.66^{a}	1.46^{b}	1.43^{b}	1.70^{a}	1.38^{b}	1.46^{b}	0.770	< 0.001	0.008
Parameters of digestive tract (n = 16)									
Proventriculus [g/kg BW]	4.53^{a}	3.59^{bc}	4.37^{a}	4.36^{a}	3.40^{c}	4.00^{ab}	0.029	< 0.001	0.711
Gizzard [g/kg BW]	17.40^{a}	11.74^{cd}	12.55^{cd}	15.56^{b}	9.25^{d}	14.30^{bc}	0.099	< 0.001	0.002
Proventriculus lengh [cm]	2.97^{b}	3.29^{a}	3.36^{a}	2.91^{b}	3.30^{a}	3.22^{ab}	0.336	< 0.001	0.623
Proventriculus width [cm]	1.84^{b}	2.11^{a}	2.09^{a}	1.88^{ab}	2.09^{a}	1.88^{ab}	0.206	< 0.001	0.076
Isthmus score	0.9^{b}	2.2^{a}	2.0^{a}	1.8^{ab}	1.7^{ab}	1.0^{b}	0.179	0.014	<0.001
Small intestine [g/kg BW]	3.20^{a}	2.63^{bc}	2.60^{bc}	2.85^{b}	2.40^{c}	2.73^{b}	0.005	< 0.001	0.001
Small intestine [cm]	179^{c}	200^{ab}	206^{ab}	189^{b}	212^{a}	202^{ab}	0.045	< 0.001	0.059

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113.

In situ ruminal starch and crude protein degradation of barley, rye and triticale grains

In situ-Abbau von Stärke und Rohprotein von Gerste-, Roggen- und Triticalekörnern

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Breeding of cereal grains leads to new varieties with potentially modified nutritive value. Textbook values for ruminal degradation characteristics of cereal grains are mostly based on data obtained more than a decade ago. Therefore the aims of this study were to identify inter- and intra-species variations of the ruminal degradation of starch (ST) and crude protein (CP) in barley, rye and triticale grains.

Methods: Twenty genotypes of each species were grown at the same location under the same agronomic conditions and the grains were analysed for a broad spectrum of physical and chemical characteristics. The ST and CP concentrations ranged between 51-58% and 11-14% for barley; 56-61% and 11-13% for rye and 57-67% and 12-13% for triticale (on dry matter basis). Grain samples were ground (2 mm) and weighed into nylon bags (50 µm pore size) for *in situ* incubations. The incubations were carried out in the time span from 0 to 48 h in 3 ruminally fistulated, lactating cows. The ST and CP concentration of the grains and bag residues were predicted using NIRS calibrations specifically developed for this matrices (1). An exponential curve was fitted for estimating the degradation parameters a (washout fraction), b (degradable fraction) and c (degradation rate). Effective ST and CP degradation for a passage rate of 8 %/h (ED_8) was calculated (2).

Results: Estimated maximum ST degradation (a+b) was almost complete in all grain species (Table). Degradation rates (c) of ST were overall very high but with considerable variation between and within grain species. Extent and rate of CP degradation was lower than for ST. Positive correlations were found between c of ST and CP using all grains ($P < 0.001$) and within rye ($P = 0.02$). The ED_8 of ST and CP was significantly lower for barley compared to rye and triticale. The highest variation in ED_8 was found within barley for CP.

Table Ruminal *in situ* crude protein (CP) and starch (ST) degradation of grains (n=20 genotypes/grain species)

			Rye		Triticale		Barley		SE
			mean	range	mean	range	mean	range	
ST	a	(%)	31[b]	23 - 35	35[a]	26 - 42	25[c]	17 - 30	0.9
	a+b	(%)	99[b]	99 - 99	99[c]	98 - 100	100[a]	99 - 100	0.1
	c	(%/h)	117[a]	77 - 177	85[b]	54 - 137	36[c]	28 - 48	5.1
	ED_8	(%)	95[a]	92 - 96	93[b]	90 - 95	85[c]	82 - 88	0.3
CP	a	(%)	32[a]	28 - 35	31[a]	23 - 37	26[b]	20 - 30	0.7
	a+b	(%)	95[c]	94 - 96	97[b]	96 - 98	98[a]	96 - 99	0.2
	c	(%/h)	43[a]	39 - 50	27[b]	21 - 32	20[c]	14 - 25	0.7
	ED_8	(%)	85[a]	83 - 86	82[b]	79 - 84	77[c]	69 - 80	0.4

a.b.c Values within a row with different superscripts differ significantly at $P < 0.05$.

Conclusion: The small variation of nutrient concentrations and ED of ST and CP within one grain species may result from the standardised cultivation conditions on the field. Differences in the ED of ST and CP between grain species should be considered in feed formulation, but variation within one grain species in estimated ED was not big. It is suggested that a mean value for the ED of ST and CP for each grain species is used in feed formulation.

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114.

Substitution of concentrates by sugar-rich hay in the diet affects ruminal ammonia concentration and nutrient digestibility in Holstein cows

Der Ersatz von Kraftfutter durch zuckerreiches Heu beeinflusst die Ammoniakkonzentration im Pansen und die Nährstoffverdaulichkeit bei Holstein Kühen.

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Introduction: Feeding dairy cows starch-rich concentrates is necessary to counteract energy deficits during lactation. High concentrate levels of 40% and more in dairy rations might lead to rumen dysfunctions (1). The inclusion of high quality forages such as sugar-rich hay might be a healthy alternative to concentrate feeding in dairy cattle. Nevertheless, a substitution of concentrates by sugar-rich hay in dairy rations might affect the energy- and protein-use efficiency through an altered energy supply and microbial activity. The aim of this study was to investigate the influence of a gradual substitution of concentrates by sugar-rich hay on the ruminal ammonia concentration, and the apparent total tract nutrient digestibility in Holstein cows.

Methods: Four different diets (DM-basis), 60% standard hay and 40% concentrate (CON), 60% sugar-rich hay and 40% concentrate (G1), 75% sugar-rich hay and 25% concentrate (G2), and 100% sugar-rich hay (G3), were fed to 8 ruminally-cannulated non-lactating Holstein cows arranged according to a double 4 x 4 Latin Square design. The sugar-rich hay and the standard hay contained on average 19 and 11% sugar as well as 46 and 58% NDF (DM-basis), respectively. Each run consisted of 25 d, with 12 d for adaptation to the test-diets, and the last 13 d for experimental measurements. Ruminal samples from the free rumen liquid (FRL) and the particle-associated rumen liquid (PARL) were taken at different time points and the ammonia concentration was measured photometrically. The apparent total tract nutrient digestibility was analyzed using TiO_2 as an external marker. Statistical analysis was performed using the MIXED procedure of SAS.

Results: The ammonia concentrations in the FRL and PARL were significantly influenced by time, diet and location ($P < 0.01$). The differences between the experimental groups were more pronounced in the PARL, showing higher values in G1 (60% sugar-rich hay/40% concentrate), G2 (75% sugar-rich hay/25% concentrate), and G3 (100% sugar-rich hay) with 20.4, 21.8, and 24.0 mmol/L. Lower ammonia concentrations were found in CON and especially in the FRL where concentrations ranged from 4.8 mmol/L in CON to 15.5 mmol/L in G3. The sugar-rich hay diets showed a higher apparent total tract digestibility of DM and OM averaging 74 and 76%, compared to CON with 65 and 68%, respectively. The digestibility of CP, NDF, and ADF linearly increased with an increasing portion of sugar-rich hay in the diet showing digestibility values ranging from 68, 60, and 53% in CON to 76, 81, and 78% in G3. The group that received 100% sugar-rich hay (G3) showed the lowest digestibility of ether extract and of the non-fiber carbohydrates.

Conclusion: Data suggest that inclusion of 75 and 100% sugar-rich hay at the expense of starch-rich concentrates increases the availability of nitrogen for rumen microbes and enhances the digestibility of DM, OM, CP, NDF, and ADF. Further research is necessary to evaluate whether and to which extend the substitution of sugar-rich hay in the diet can compensate the energy and nutrient deficits of early-lactating cows.

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115.

The effects of microbial fermentation and enzymatic pre-digestion of pea on performance and nutrient digestibility in broilers

Der Einfluss einer mikrobiellen Fermentation und enzymatischer Vorverdauung von Erbsen auf Leistung und die Nährstoffverdaulichkeit bei Broilern

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Peas are legume crops with moderately high protein and starch content. The anti-nutrients in pea have limited its use in poultry diet as a protein source. Fermentation can reduce the negative impacts of anti-nutrients in legumes (1). The major beneficial impact of fermentation processes on anti-nutrients seems to be through enzymatic pre-digestion. Supplementation of enzymes in poultry diets in order to degrade anti-nutrients in feed and increase the availability of nutrients are a common practice in poultry production. However, the relatively short retention time of digesta in broiler gut and the wide range of pH which feed encounters along the gut limit the efficiency of additive enzymes (2).

Fermentation of pea with probiotics and pre-digestion of pea for a certain period of time with appropriate enzymes may offer an interesting perspective to improve the nutritional quality of pea products. The present study examined the impact of different inclusion levels of raw, fermented or enzyme treated peas on performance and nutrient digestibility in broilers.

Methods. For fermentation process, Madonna pea, at the ratio of 1:1, was mixed with water containing 2.57 x 10^8 Bacillus subtilis (GalliPro®, Chr. Hansen, Denmark) spores/kg pea and then fermented for 48 h at 30°C. For the enzymatic pre-digestion, the pea, at the ratio of 1:1, was soaked in water containing three commercial enzymes including AlphaGal™ (0.1 g/kg pea - Kerry EMEA, USA) containing an α-galactosidase, RONOZYME® ProAct (0.2 g/kg pea - DSM, Switzerland) containing a protease, and RONOZYME® VP (0.2 g/kg pea - DSM, Switzerland) containing pectinases, and then incubated for 24 h at 30°C. In a two-factorial design, nine corn-wheat-soybean diets were formulated by supplying 10, 20 and 30% of the crude protein with the 3 different pea products (about 100, 200 and 300 g/kg feed in starter diets and 90, 180 and 270 g/kg feed in grower diets) including raw pea, fermented and pre-digested pea. One thousand and eighty 1-day-old broilers were assigned to 72 pens (8 pens per diet). The performance variables of broilers were recorded weekly. At d 35, the ileal digesta of 9 birds per pen were collected, pooled and used for the apparent ileal digestibility analysis. Data were subjected to ANOVA using the GLM procedure with a 3 × 3 factorial arrangement of treatments. The treatment means were separated by the Tukey test at $P \leq 0.05$ statistical level.

Results. Both types of processes caused reduction in the raffinose equivalents, trypsin inhibitor activity and resistant starch.

Increasing inclusion level of pea products reduced body weight gain and feed intake. The birds fed enzymatic pre-digested pea had the best feed efficiency at d 35 ($P \leq 0.05$). Both types of processes had an identical effect on ileal digestibility of protein, fat, phosphorus, calcium, potassium and amino acids. The ileal digestibility of starch in diet with raw peas was lower compared with fermented and pre-digested pea ($P \leq 0.05$). The inclusion levels of pea products had no effect on ileal digestibility of most nutrients (except starch, Thr, Lys and Met). However, at 30% inclusion level of peas the apparent ileal digestibility of Thr, Lys and Met were higher compared with the 10% inclusion group ($P \leq 0.05$), while chicken fed only 10% pea products showed highest apparent ileal digestibility of starch ($P \leq 0.05$).

Conclusion. The present study demonstrated that fermentation and enzymatic pre-digestion processes could relatively improve the nutritional quality of peas. Moreover, replacement of soybean meal by pea derived products at less than 20% inclusion level in the diet might have no negative impact on the nutrient digestibility and broiler growth performance. These findings indicate the feasibility of fermentation and enzymatic pre-digestion processes for improving the nutritional quality of pea as a partial replacement for soybean meal in broiler diets.

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116.

Effects of a varying structural value of diets for fattening bulls on feed intake and fattening performance

Einfluss eines variierenden Strukturwertes in Rationen für Mastbullen auf Mast- und Schlachtleistung

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Adequate supply of structural fibre in the diet of ruminants is essential to maintain normal ruminal function and to prevent Subacute Ruminal Acidosis (SARA; 1). However, in contrast to dairy cows in which adequate supply of structural fibre is presently in focus, there is only scarce information on adequate fibre supply in fattening bulls. For Belgian blue bulls the minimum structural value (SV) of diets is estimated to be 0.6 (2), what appears to be very low compared to requirements of dairy cows. In order to re-evaluate those data, a feeding trial was conducted to investigate effects of diets varying in structural value on performance of fattening bulls in the finisher phase.

Methods: 70 growing German Simmental bulls (Body weight (BW): 517 ±32 kg; age: 314 ±11days (d)) were assigned equally to one of three dietary treatment (treat) groups. Treat SV 1.2 were fed for ad libitum intake a Total Mixed Ration (TMR) based on maize silage (65 % of dry matter (DM)), straw (4 % of DM), and concentrates (31 % of DM). For treat SV 1.1, straw was removed from this TMR. Treat SV 0.6 was fed a TMR composed of 30 % maize silage and 70 % concentrates in DM. Diets were calculated to be comparable in all nutrients except of structural fibre (peNDF > 1.18, g/kg DM: 294, 270, and 246 for diets SV 1.2, SV 1.1, and SV 0.6, respectively), which was enabled by reduced amounts of cereals and increased amounts of dried sugar beet pulp in concentrates for treat SV 0.6. Individual feed intake was automatically recorded daily while BW was recorded every four weeks. Reticuloruminal pH was measured by an indwelling and wireless data transmitting unit (smaXtec pH & Temp Sensor; smaXtec animal care sales GmbH, Austria). Eight animals of each treat were equipped with the sensor and a recording time of 38 d was evaluated, resulting in a total of 304 pH-test-days per treat. The bulls were slaughtered at an average age of 505 days. The data were evaluated by a one-factorial ANOVA using SAS. Data of 23, 22, and 22 animals were used for groups SV 1.2, 1.1, and 0.6.

Results: Daily DM, ME and starch/sugar intake increased when SV of the diet decreased (table). End weight and daily gain tended to increase with lower levels of SV. Animals in group SV 0.6 tended to have higher risk for SARA when ruminal ph borderline values for dairy cows are considered.

Treatment:	SV 1.2	SV 1.1	SV 0.6
Feed intake, kg DM/d	10.7±0.9^{b}	11.2±1.1^{b}	12.0±1.0^{a}
Energy intake, MJ ME/d	125±11^{b}	131±13^{b}	144±12^{a}
Starch+Sugar intake, g/d	3481±295^{c}	3798±372^{b}	4496±384^{a}
aNDFom intake, g/d	3662±306	3657±361	3798±330
Final body weight, kg	798±52	797±70	817±65
Daily gain, g	1480±170	1457±288	1548±221
Days with pH < 5.8 for > 5.2 h, n	38	39	46
Days with pH < 6.15 h, n	5	5	10

Conclusions: Feed intake and performance data indicate that fattening bulls in the finishing phase can be fed TMR with a low SV of 0.6. Measurement of ruminal pH indicates a higher risk for SARA when SV of TMR is decreased to 0.6, but low ruminal pH-values appear to be scarce compared to dairy cows. This will be due the fact that pH was measured in the reticulum, but not in the ventral sac as in most studies with dairy cows. Moreover, this may be as a result of lower feed intake in fattening bulls relative to dairy cows and a lower ruminal acid load.

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117.

Effects of ethyl esters on preference behaviour of goats

Einfluss von Ethylestern auf das Futteraufnahmeverhalten von Ziegen

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Introduction: Whole-crop maize silages with atypical smell and decreased feed intake by ruminants have shown to contain high concentrations of volatile organic compounds (VOC), especially the esters ethyl lactate (EL) and ethyl acetate (EA) in a strong positive correlation to the ethanol (EtOH) content (1). It is assumed that they may negatively influence feed intake by impacting sensory silage characteristics.

Methods: Three preference trials (15/21/21 days) with goats (Saanen type goats; n = 10/n = 5/n = 5) were conducted. A maize silage with good fermentation quality and grass hay were used as substrate, which was supplemented with different dosages of EL and EA (Table 1), either alone or in combination with EtOH. The amount of the added VOC was based on concentrations naturally occurring in maize silages. The VOC were diluted with water and applied homogenously with a spray bottle to the forages which were mixed thoroughly afterwards. Each trial started with an adaptation period where all forages were fed as single meal. During the experimental period of each trial, forage treatments were offered in all possible two-way combinations as free choice for 3 h. Statistical analyses were performed using one-factorial ANOVA, treatment differences were assessed by Waller-Duncan *k*-ratio *t*-test.

Results: Results of the three trials do not show a clear effect of supplemented ethyl esters on preference behaviour (Table 1). In trial 2 und 3, dry matter intake was constant for all variants irrespective of ester concentration. Based on these data, modifications in the sensory characteristics caused by formation of ethyl esters alone might not be the primary cause for reduced feed intake. Both ethyl esters were recently mentioned in connection with metabolized mycotoxins in maize silages (2) which can be seen as another possible explanation for avoidance of those silages.

Table 1: Dry matter intake (DMI; g/3 h) of forages supplemented with ethyl lactate (EL), ethyl acetate (EA) and ethanol (EtOH) in three preference trials with goats (dosage in mg/kg DM)

1) Substrate maize silage (n = 40/treatment)		2) Substrate grass hay (n = 25/treatment)		3) Substrate grass hay (n = 25/treatment)	
Treatment	DMI	Treatment	DMI	Treatment	DMI
Control	745[a]	Control	567	Control	528
560 mg EL	508[b]	600 mg EA	522	30 g EtOH + 600 mg EA	515
910 mg EL	651[a,b]	600 mg EL	544	30 g EtOH + 600 mg EL	534
1290 mg EL	723[a]	1200 mg EA	550	60 g EthOH + 1200 mg EA	461
		1200 mg EL	532	60 g EtOH + 1200 mg EL	475
		600 mg EA + 600 mg EL	561	60 g EtOH + 600 mg EA + 600 mg EL	480

[a,b] Values within a column having different superscripts differ significantly ($P<0.05$)

Conclusions: In three preference trials, ethyl esters added to different forages have not altered feeding behaviour and short-time dry matter intake of goats. On-farm observations of reduced intake when silages high in ester concentrations are fed may therefore indicate that not esters alone but, more likely, a combination of VOC or substrates are responsible. Adding single VOC to forages does not reflect the total factors responsible for reduced intake of silages with atypical smell.

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118.

Partitioning of dietary nitrogen in lactating dairy cows fed different proportions of red clover silage in the diet

Verteilung des aufgenommenen Futterstickstoffs bei laktierenden Milchkühen bei Fütterung verschiedener Rotkleesilageanteile in der Ration

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Question: High amounts of non-protein nitrogen as well as rapid and extensive ruminal degradation of proteins in legume and grass silages generally lead to poor efficiency of nitrogen (N) utilization (milk N output/N intake) and high urinary N excretion. Distribution between faecal and urinary N in manure is of particular interest, because urinary N is much more susceptible to gaseous losses and leaching. Due to the action of o-quinones formed by polyphenol oxidase, protein breakdown during ensiling and rumen fermentation is supposed to be less extensive in red clover. Hence, red clover silage (RCS) might have the potential to improve efficiency of N utilization and also to enhance supply of undegraded crude protein from forage. The aim of this study was to investigate the effect of incremental replacement of maize silage (MS) and soybean meal (SBM) by RCS (2nd cutting) and wheat on the partitioning of dietary N in dairy cows.

Methods: 44 primi- (n=18) and multiparous (n=26) Holstein-Friesian cows were used in a 4x4 Latin square trial with 21-d periods (13 d adaption followed by 8 d sample collection). Cows averaged 38.7±7.3 kg/d milk, 1.9±1.1 lactations, and 149±103 days in milk at the start of the trial. Treatments comprised total mixed rations containing about 74% forage (DM basis) with proportions (% of DM) of RCS:MS in the diet of 14:61 (diet RCS_{14}), 27:47 (diet RCS_{27}), 42:32 (diet RCS_{42}), and 57:16 (diet RCS_{57}). All diets contained 9% (DM basis) lupine seeds, whereas wheat gradually replaced SBM with increasing dietary proportion of RCS to obtain isonitrogenous diets. Thus, diet RCS_{14} contained 16% SBM (DM basis), and diet RCS_{57} included no SBM. During each sampling phase, faecal grab samples (d 15, 17, 19, and 21) and spot urine samples (d 14, 16, 18, and 20) were taken and analysed for N. Samples for milk composition were collected daily. To estimate faecal excretion, 30 g of the external marker titanium dioxide were dosed orally once a day to cows during d 10 to 20 of each period. Urinary N excretion was calculated as N intake - (faecal N + milk N), neglecting possible changes in body N. Mixed model procedure followed by ANOVA and Tukey tests were conducted.

Results: Since dietary treatment affected N intake ($P<0.001$) through effects on DM intake ($P<0.001$; data not shown), N outputs in milk, faeces, and urine should be compared when expressed as percentages of N intake. Cows fed diet RCS_{57} produced less ($P\leq0.004$) milk N than cows consuming the other three diets. Faecal N increased ($P<0.001$) when diets with greater proportions of RCS (plus wheat at the expense of MS plus SBM) were fed instead of diet RCS_{14}. Cows fed diet RCS_{42} excreted less ($P<0.001$) N in urine than cows consuming the other three diets.

	Dietary treatment					
Parameter	RCS_{14}	RCS_{27}	RCS_{42}	RCS_{57}	SEM	P-value
N intake, g/d	628^{a}	611^{a}	553^{b}	561^{b}	18.7	< 0.001
Milk N, g/d	178^{a}	169^{b}	154^{c}	140^{d}	3.24	< 0.001
Faecal N, g/d	198^{b}	210^{ab}	214^{a}	208^{ab}	7.32	0.008
Urinary N, g/d	252^{a}	232^{ab}	184^{c}	212^{b}	11.3	< 0.001
Milk N, % of N intake	29.0^{a}	28.0^{a}	28.4^{a}	25.4^{b}	0.95	< 0.001
Faecal N, % of N intake	31.6^{d}	34.3^{c}	38.8^{a}	37.2^{b}	0.40	< 0.001
Urinary N, % of N intake	39.5^{a}	37.7^{a}	32.8^{b}	37.4^{a}	0.85	< 0.001

$^{a-d}$ Least square means within the same row with different superscripts differ at $P<0.05$.

Conclusions: Under the conditions of this study, efficiency of N utilization was maintained when RCS and wheat partially replaced MS and SBM in the diet. Simultaneously, N excretion was shifted from urine to faeces, which may reduce negative effects on the environment.

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119.

Effect of feeding differently processed peas on lymphocyte subpopulations of the gut-associated lymphoid tissue in broilers

Einfluss der Fütterung unterschiedlich behandelter Erbsen auf die Lypmphozytensubpopulationen des darmassoziierten lymphatischen Gewebes bei Broilern

*Röhe I., Goodarzi Boroojeni F., Zentek J. – Berlin

Peas are a traditional protein source for poultry. By using different processes such as enzyme treatment or fermentation the nutritional value of peas can be improved leading to a reduction of pea associated antinutritional factors (1), which are related to immunomodulating effects (2). Hence, the current study aims to proof the effect of feeding differently processed peas on the intraepithelial lymphocyte subpopulations in the jejunum of broilers (for performance and nutrient digestibility data, please see the abstract from Goodarzi Boroojeni in this issue).

Methods: One-day-old broilers were allocated to four different experimental groups of 15 birds per pen. Birds were fed with one of the following mash diets over a period of five weeks: A control diet, based on corn and soybean meal (C), as well as three treatment diets containing raw pea (RP), fermented pea (FP) and enzymes-treated pea (EP), each supplying 30% of diet's crude protein. In total, six replicates were performed. At the end of each trial run, broilers were killed and jejunal samples taken for immunohistochemical (IHC) as well as flow cytometric (FC) analyses. IHC analyses were focused on the determination of $CD45^+$ intraepithelial lymphocytes (IEL) as well as epithelial $CD3^+$ T- cells. T cells and their subpopulations, NK cells and B cells were detected by FC analyses. Due to normal distribution, statistical analyses were conducted by ANOVA and posthoc Tukey's testusing SPSS (version 22.0, Chicago, IL, USA).

Results: The IHC analyses showed differences in the number of $CD45^+$ and $CD3^+$ IEls (Table 1): The treatment groups had higher numbers of $CD45^+$ IEls in the tip- ($P = 0.004$) and mid- ($P < 0.001$) villus region compared to birds fed with C. Furthermore, increased numbers of $CD3^+$ cells were found in the villus tip- ($P = 0.002$) and mid- ($P = 0.003$) region of birds receiving RP, FP and EP in comparison with C. No differences in IEL numbers were found in the crypt regions. The FC analyses revealed no differences in the relative distribution and frequency of IELs among the feeding groups.

Table 1 Number of jejunal $CD45^+$ and $CD3^+$ lymphocytes/100 epithelial cells in the tip- and mid- region of villi as well as in crypts/10.000 µm²of broilers fed with the different diets

IEL	Region	C[1]	RP	FP	EP	P-value
$CD45^+$	Villus tip	$22.2^a \pm 8.12$	$32.8^b \pm 4.85$	$32.6^b \pm 3.82$	$35.4^b \pm 4.40$	0.004
	Villus mid	$32.3^a \pm 8.25$	$48.7^b \pm 6.43$	$47.1^b \pm 2.66$	$49.0^b \pm 5.26$	< 0.001
	Crypt	16.6 ± 2.95	19.4 ± 3.21	21.5 ± 1.57	22.0 ± 5.26	0.075
$CD3^+$	Villus tip	$13.3^a \pm 4.67$	$21.9^b \pm 2.24$	$21.2^b \pm 4.12$	$23.0^b \pm 4.01$	0.002
	Villus mid	$20.1^a \pm 5.22$	$31.6^b \pm 5.44$	$30.2^b \pm 4.42$	$30.7^b \pm 4.98$	0.003
	Crypt	6.61 ± 3.96	12.0 ± 5.82	11.0 ± 3.05	11.7 ± 4.31	0.165

Means with different superscripts are significantly different ($P < 0.05$);
Results are reported as means of 6 replicate pens ± SD;
[1] Animals fed with: C=Control diet; RP= raw pea diet; FP= fermented pea diet; EP= enzymes-treated pea diet.

Conclusion: The implementation of peas in the broiler diets resulted in an increase of jejunal IELs suggesting an immune modulating effect of pea containing components. Neither fermentation nor enzyme treatment of peas influenced the frequency of lymphocytes in the jejunal epithelium. Whether e.g. antinutritional factors or the pea protein itself might be responsible for this effect needs further clarification.

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120.

A new grinding technology for soybean byproducts in broiler diets: effects on performance, excreta and litter quality as well as on foot pad health

Bedeutung besonders fein vermahlener Sojaprodukte im Mischfutter für die Leistung, Exkremente- und Einstreu-Qualität sowie für die Fußballengesundheit bei Broilern

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Soybean byproducts are an important protein source in broiler diets. Approaches are described, that not all components of a diet should be ground finely (for higher digestibility), but left coarsely (for an improved gut health2). Yet, it is still on debate whether protein rich ingredients should be separately ground finely to improve digestibility of proteins and amino acids3.

The aim of this study was to investigate the effects of differently ground soybean products [Low-Protein-Soybean meal (LP-SBM), High-Protein-Soybean meal (HP-SBM) and Soy Protein Concentrate (SPC)] in broilers' diets on performance, excreta quality and on foot pad health.

Methods: At the age of 7 days 240 broilers (Ross 708, male) were divided into 10 groups. The pelleted diets, based on wheat and corn, and 3 different protein sources (mentioned above) were used. Each soybean byproduct was available in a conventionally ground version (2-20% of particles passed a 200 µm sieve in a modified dry sieve analysis) but also in a very fine particle size (mean of particles < 63 µm; more than 98 % of particles passed a 200 µm sieve) due to a new grinding technology.

Group	1	2	3	4	5	6	7	8	9	10
Soy product	LP-SBM				HP-SBM			SPC		
Conventionally ground protein source, in % of used soy products	100	67	34	0	100	50	0	100	50	0
Finely ground protein source, in % of used soy products	0	33	66	100	0	50	100	0	50	100

Feed and water consumption were measured daily on group basis. FPD-Scores4 and individual body weight (BW) were measured weekly. Moreover, excreta and litter samples were taken weekly as pooled samples per group to analyse the DM content. Statistical analyses were performed by using SAS® software (Cary, NC, USA) (PROC GLM/PROC NPAR1WAY).

Results: The following table summarizes the most important results of the study:

Soybean	LP-SBM				HP-SBM			SPC		
Group (n)	1 (30)	2 (30)	3 (30)	4 (29)	5 (15)	6 (15)	7 (15)	8 (15)	9 (15)	10 (15)
Diet´s particle size mass (%), <200 µm	43.4	45.9	48.9	54.3	40.3	48.5	45.5	44.1	43.6	51.1
BW, d 35 (g)	1885^{a} ±397	2047^{ab} ±329	2089^{b} ±360	2215^{b} ±342	1928^{a} ±382	1987^{a} ±365	1931^{a} ±368	2155^{a} ±299	2107^{a} ±204	2154^{a} ±304
Total FCR (d 7-35)	1.48	1.48	1.38	1.46	1.50	1.47	1.45	1.43	1.46	1.46
Excreta, DM, %	17.5	17.5	17.5	14.5	17.4	17.0	16.7	15.8	15.0	14.0
Final litter, DM, %	43.0	38.9	37.2	40.2	43.8	43.4	38.4	34.3	35.8	34.9
FPD-Score, d 35	2.95^{a} ±0.79	3.14^{ab} ±0.85	3.29^{b} ±0.62	4.10^{c} ±0.67	2.63^{a} ±0.89	3.58^{b} ±0.76	3.67^{b} ±0.48	4.32^{a} ±0.48	4.19^{a} ±0.49	4.37^{a} ±0.61

The new grinding technology resulted in favourable effects on performance when LP-SBM was used. Regarding HP-SBM and SPC the new, high grinding intensity did not result in higher performance but there was a trend for unfavourable changes in excreta/litter quality as well as on foot pad health when only finely ground soy products were used.

Conclusion: The grinding intensity had an influence on performance as well as on severity of FPD, when LP-SBM and HP-SBM were used (grinding altered particle size more marked than in previously "fine" SPC, e.g.). In further investigations the effects on the digestibility of protein and amino acids is going to be tested as well as effects on digesta quality.

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121.

An artificial infection of weaned piglets with a defined *E. coli* strain (F4, STI, STII, LTI) and its effect on intestinal secretion and absorption of electrolytes

Die Effekte einer experimentellen Infektion von Absetzferkeln mit einem spezifischen E. coli-Stamm auf die intestinale Sekretion und Absorption von Elektrolyten

*von und zur Mühlen F., Sander S. J., Verspohl J., Kamphues J. – Hanover

Introduction: One of the most obvious diseases in newly weaned piglets is diarrhoea caused by *E. coli*. This pathogenic agent induces secretory diarrhoea with stimulating the secretion and reducing the absorption of sodium (Na) and chloride (Cl) in the caudal small intestine [1]. Besides the dietary use of organic acids to protect piglets against the pathogens [2], feeding a coarsely ground meal diet had an effect on the duration of shedding the agent as well as on the period with lowered faecal dry matter (DM) content after an artificial infection [3]. Point of interest in the investigations now was the effect of an artificial infection with *E. coli* on the concentration of electrolytes in faeces and the influence of diets' physical form (meal/pellet) on it. Furthermore, the question was whether faecal electrolyte content in combination with faecal DM-content can be used as a diagnostic tool for identifying secretory diarrhoea.

Material: One trial was performed with 20 weaned piglets (n=10 per group; 6.06 ± 0.32 kg BW). The test diets differed in compaction (CP: 216/218g; ME: 13.5/13.6MJ; Na: 1.25/1.35g; Cl: 3.27/3.50g; K: 9.07/8.89g per kg diet as fed): a coarsely ground meal diet (CM_D) and a coarsely ground pelleted diet (CP_D). Both diets had a chemically and botanically identical composition, were offered once a day and fed ad libitum. Feed intake, a faecal score (1=solid, formed; 2=mellow, formed; 3=fluid, unformed; 4=skilly; 5=watery) and faecal DM-content were recorded daily. After 20 days of feeding the test diets, each piglet was artificially infected with an orally applied dose of 1.86×10^{10} cfu of a defined *E. coli* strain (F4, STI, STII, LTI) known to induce severe diarrhoea in weaned piglets. From 2 days prior to 6 days after the artificial infection (d18-d26 of trial) the concentration of sodium, potassium and chloride was measured daily in the faeces. Statistical analyses were done using the SAS 9.3 software (analysis mixed models, respectively, $p \leq 0.05$).

Results: Animals' performance as well as the counts of the applied *E. coli* excreted via faeces did not differ between the groups. But piglets fed the CM_D diet showed a shorter period of shedding so that only in 10 (CM_D) vs. 30% (CP_D) of individual faeces samples the applied *E. coli* was detectable on d26.

$\bar{x} \pm sd$		d20			d20.5			d21			d22		
DM-content	**CM_D**	249^a	±	29.6	151^b	±	19.9	231^a	±	43.6	219^a	±	55.7
(g/kg FM)	**CP_D**	272^a	±	16.5	165^b	±	39.8	225^a	±	56.0	241^a	±	51.6
Na in faeces	**CM_D**	2.23^a	±	1.35	8.91^b	±	3.58	1.56^a	±	1.17	2.36^a	±	2.14
(g/kg DM)	**CP_D**	1.99^a	±	1.10	9.87^b	±	3.22	2.57^a	±	2.13	1.90^a	±	1.79
Cl in faeces	**CM_D**	2.15^a	±	1.31	5.86^b	±	2.03	1.28^a	±	1.33	4.24^{ab}	±	4.34
(g/kg DM)	**CP_D**	1.61^a	±	0.50	4.35^b	±	2.03	1.88^{ab}	±	2.10	2.87^{ab}	±	2.00
K in faeces	**CM_D**	16.1^a	±	4.68	16.1^a	±	4.76	17.8^a	±	4.78	19.6^a	±	6.90
(g/kg DM)	**CP_D**	15.8^a	±	1.83	16.1^a	±	4.44	18.8^a	±	3.12	19.9^a	±	4.19

d20: day of artificial infection; d20.5: 8-12h after artificial infection; $^{a, b}$ $p \leq 0.05$ in a line;

Conclusion: Some hours after infection, sodium and chloride concentrations in faeces were 7.6 or 2.7 times higher than before infection but this effect was not seen on d21 any longer and there was no significant difference due to diets' physical form. Same was observed with faecal DM-content. That might be explained by piglets' age - being weaned already four weeks before artificial infection and with this the capacity to balance intestinal electrolyte and water contents was higher. So it is not clearly identifiable whether faecal electrolyte concentration reflects secretion in the small intestine. Although, the significant increase in faecal electrolyte content shortly after oral uptake of the pathogenic agent indicates a significant increase in electrolyte losses and the use as diagnostic tool.

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Effects of different dietary protein sources on performance, litter quality and foot pad health in broilers

Einfluss unterschiedlicher Proteinkombinationen im Alleinfutter für Broiler auf die Leistung, die Einstreuqualität und die Fussballengesundheit

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Question: Soybean meal is the current main source of protein for poultry feed over the world. Especially in countries that import the majority of feed materials, alternative protein ingredients are increasingly of interest. Finally, sustainability is an important driver for using alternative sources of protein. The aim of this study was to evaluate potential effects of specific combinations of protein sources on performance, litter quality and health of foot pads in broilers.

Methods: One trial was done with four groups (one replication/group) of 1-wk-old broilers, which were reared for further 37 days. All groups (20 birds in each at start; 2 qm / box) were littered with wood shavings (1 kg/m^2). Overall four different diets were fed up to dissection (d 44 of life). These were a self-made compound feed with four protein sources and combinations (4x2 groups; soybean meal=SBM; rapeseed meal=RS [WISAN®Raps]; haemoglobin meal= HBM; Algae Powder=ALG) and soyabean oil. Body weight, moisture contents of excreta and litter were measured weekly. External assessment of foot pads [1] was done weekly with scores of 0-7 (0 = normal skin; 7 = > half of foot pad necrotic). Statistical analysis was performed with SAS for Windows. To compare the body mass an analysis of variance was performed with comparison-related error probability (LSD-test); foot pads were compared using Kruskal-Wallis test followed by pairwise comparisons with Wilcoxon test ($p < 0.05$).

Table 1: Experimental set-up in terms of ingredients (protein type etc.) and feed composition

group	SBM	RS	HBM	ALG
SBM/added protein type (%)	32.5/-	22.9/14.5	22.5/4.5	28/4.0
ME (MJ/kg diet)	12.1	12.0	12.7	12.6
XP/XL (g/kg diet)	208/71.8	205/75.9	213/75.4	211/80.2
Ca/P (g/kg diet)	12.5/4.5	13.3/6.4	14.2/6.2	12.9/6.1
Na/K (g/kg diet)	1.8/8.8	1.9/8.0	1.8/6.9	1.8/8.3

Results: Losses of birds up to d 44 did not differ markedly between groups. Significant differences were found in final body weight on d 44 with significant lower body weights in group HBM. The results also revealed that foot pad dermatitis (FPD) severity was significant higher for birds fed RS and ALG diets compared with those fed SBM or HBM diets. The animals of the group RS had the numerically highest absolute feed intake. Therefore, the absolute water uptake was also very high. These higher amounts of liquid were added via excreta to the litter. In the algae group, however, the feed intake was not different from the SBM group, which had a significantly better foot pad health. But in the algae group there was a somewhat higher water:feed intake ratio. Prevalence of high FPD scores (6-7) were 76 % and 88 % for birds fed rapeseed meal (RS) and algae diets (ALG) vs. 46 % and 56 % for those fed control (SBM) and haemoglobin powder meal diets (HBM), respectively.

Table 1: Effects of dietary protein sources on BW, litter quality and foot pad health in broilers

group	SBM	RS	HBM	ALG
final BW at d 44 (g)	2568^{ab}±277	2726^{a}±310	2345^{c}±291	2545^{b}±333
feed conversion ratio d 7 - d 44	1.83	1.84	1.78	1.77
water:feed intake ratio	2.53	2.49	2.53	2.68
excreta DM (%, mean)	15.6±2.5	16.4±2.1	15.7±2.7	14.6±2.0
„final litter“ - DM (%)[1]	44.8	46.6	46.5	47.8
FPD scores at d 44	5.42^{b}±1.21	6.28^{a}±1.02	5.48^{b}±1.54	6.58^{a}±0.01

[1]whole litter material (wood shavings, excreta, feathers…)

Conclusion: It seems that using RS and ALG diets could partially replace the soybean meal without any negative effects on body weight gain. The higher severity of FPD in birds fed RS might be related to the high overall feed intake and therefore a higher water turnover in boxes. The phenomenon of a higher water intake in the ALG group was also seen by other researchers [2] but up to now it can only be speculated if certain ingredients are responsible for that (complex carbohydrates, minerals, etc.). Biotin and zinc have a known positive impact on foot pad health. Algae are known for their somewhat higher Biotin- and Zn-content. Possible positive effects might be masked the specific ingredients mentioned above. Influences of fatty acid patterns on FPD have not been part of the investigation, but should be considered in future studies.

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123.

Effects of *ad libitum* milk intake on sucking behaviour, performance and faeces quality in calves within first days of life

Effekte einer ad libitum Tränke auf Saugverhalten, Leistung und Kotqualität von Kälbern in den ersten Lebenstagen

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An *ad libitum* offer of a milk replacer to newborn calves is a controversial issue. On the one hand it is argued that an intense growth during rearing has positive effects on the future dairy cow's milk production. On the other hand, there are concerns regarding a reduced faeces quality due to a lower digestion capacity within the first days of life and economic aspects as well. Therefore, the aim of the study was to evaluate effects of an *ad libitum* intake of a milk replacer given by an automatic calf feeder on sucking behaviour, weight gain and faeces quality in calves within first two weeks of life.

Methods: The study was performed using Holstein-Friesian calves (n = 47) of a dairy herd. After administration of a farm's own mixed colostrum, animals were randomly allocated to three groups and housed individually in calf hutches (straw bedding). A commercial milk replacer (200 g/L, 22.5% XP, 16.5 MJ ME/kg) was fed to calves individually and *ad libitum* using an automatic calf feeder (group 1; G1), whereas in group 2 (G2) the milk replacer was offered restrictively (3 x 2.5 L/d) in buckets with a teat. Calves of group 3 (G3) were fed with acidified whole milk (2 x 8 L /d) using buckets with a teat as well. Individual feed intake was daily quantified in all groups. From the seventh day of life, hay and concentrate were offered additionally (daily determination of the ingested amounts). During the first feed offer in group 2, the sucking behaviour was observed simultaneously in all groups. Body weights of the calves were measured using a mobile scale and faeces quality was evaluated by means of a score (on day 1, 7 and 14 of life). The classification was made according to mushy pasty (1), mushy thin (2), creamy thin (3) and watery thin (4) and the dry matter (DM) content was analysed. Statistical evaluation of results was performed using SPSS (version 20).

Results: Feed intake, sucking behaviour, body weight gain and faeces quality in calves within the first two weeks of life are shown in the following table:

		G1	G2	G3
feeding system feed		automatic calf feeder milk replacer	bucket with teat milk replacer	bucket with teat whole milk
offered amount		ad lib.	restr. (3 x 2.5 l)	ad lib. (2 x 8.0 l)
drinking quantity (L/d) (min - max)		$8.84^{a} \pm 3.74$ (2.9 - 21.0)	$6.48^{b} \pm 1.44$ (2.0 - 7.5)	$7.84^{ab} \pm 3.09$ (0.2 - 16.0)
speed of drinking[1] (L/min)		0.593 ± 0.171	0.900 ± 0.938	0.572 ± 0.642
sucking process[1,2] (min/meal)		5.83 ± 1.66	2.47 ± 0.79	6.44 ± 2.48
body weight gains (g/d, 1st week)		$1401^{a} \pm 535$	$1113^{b} \pm 369$	$1316^{ab} \pm 401$
faeces quality	- score	2.10 ± 1.08	1.67 ± 0.89	2.07 ± 0.86
	- DM (%)	21.5 ± 9.90	19.9 ± 8.41	20.5 ± 7.79

[1]at 4th day of life, [2]from the beginning until the end of the sucking activity with milk intake
Means with different superscripts within in a row differ significantly ($P<0.05$).

The highest amounts of milk replacer of up to 21 L/animal and day were consumed using the automatic calf feeder. Thereby, when milk was offered *ad libitum*, drinking speed was generally slower compared to the restrictive offer of milk three times a day. Moreover, the lowest DM contents of faeces were observed in calves fed restrictively.

Conclusion: Offering a milk replacer *ad libitum* by an automatic calf feeder, calves drank a multiple amount compared to calves with a lower offer of milk as it is commonly practiced. At the same time, these animals showed the most favorable faeces qualities with the lowest rate of diarrhea, which can be explained by frequent and concurrently slow suckings at the calf feeder. The future will show, whether the calves with the higher body weight gains within the first 14 days of life will be the higher yielding dairy cows (e.g. in the first lactation).

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124.

Systems for evaluation of structural effectiveness of mixed rations for dairy cows - an on farm comparison

Ein Vergleich von Strukturbewertungssystemen für Milchkühe auf Praxisbetrieben

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Introduction: A sound prediction of structural fibre supply to dairy cows from mixed rations is of increasing importance to safely exclude production diseases like subacute rumen acidosis (SARA). Therefore, a system to evaluate structural effectiveness of rations is to be incorporated in future feeding recommendations. The peNDF system was suggested as the new German system (1). In the present study, mixed rations of farms were evaluated for their structural effectiveness using the different systems "Structure Value" (SV), "structure effective Crude Fibre" (seCF) and "physically effective Neutral-Detergent Fibre" ($peNDF_{<8}$ and $peNDF_{<1.18}$) (1). Based on the estimated fulfilment of recommendations by the respective ration (as percentage of recommendation), differences in the prediction between the systems were approached. In addition, the relation between the fulfilment of recommendation and indicators of adequateness of dietary regime was investigated.

Material and methods: Mixed rations of 40 farms (Lower-Saxony; areas of Stade and Göttingen) were evaluated. Farms were visited on 2 consecutive days and intake of the group was evaluated for one day (including quantification of residuals). Samples of the mixed ration were collected and analysed for particle size distribution (Penn State Particle Separator) and chemical composition (neutral-detergent-fibre, crude fibre, crude protein, ether extracts, starch, sugar). In addition, milk yield, protein and fat (fat/protein ratio; proportion of individuals <1.0) was evaluated for the respective herd. For 10 individuals of each herd, further indicators for appropriateness of feeding were evaluated (faecal score; net-acid-base-excretion (NABE); rumination rate as chews per minute; estimated energy balance). Data were statistically evaluated with a mixed model (dependent factor: fulfilment of structural fibre recommendations; fixed factors: structure evaluation system; proportion of concentrate in ration; proportion of maize silage in roughage). The relation to indicators was evaluated via partial regression.

Results: Test diets had an average milk yield of 34.0 L/d (min/max: 27/42), milk fat was 4.04% (3.46/4.61), concentrate proportions 32-49% and maize silage proportions of roughage 0-82%. Fulfilment of requirements was estimated highest for SV and lowest for seCF and $peNDF_{>1.18}$ (Tab. 1). Correlations for indicators like milk fat/protein ratio were found for peNDF and SV, but not for seCF.

Tab. 1: Structural fibre contents, proportions of recommendations and relation to indicators

	SV	seCF	$peNDF_{>1.18}$	$peNDF_{>8.0}$
Average value	1.59±0.29 /kg DM	2.79± 0.44 kg DM/d	31.4± 3.2% DM	19.8± 4.1% DM
Fulfilment of recommendation				
Mean, %	159 ± 32[a]	107 ± 17[b]	117 ± 37[bc]	124 ± 61[c]
Median (Min/Max), %	150 (109/232)	106 (75/151)	101 (84/274)	101 (58/234)
Relation with indicators (p-values)				
Fat/Protein ratio (% herd <1.0)	**0.0003**	0.2775	**0.0229**	**0.0073**
Chewing rate (rumination)	**0.0573**	0.5349	**0.0311**	**0.0070**
NABE	0.4209	0.5706	0.3120	0.2423
Faecal score	0.2314	0.2046	0.4590	0.2902
Estimated energy balance	**0.0292**	0.3257	**0.0002**	**0.0008**

Conclusions: The study confirms results that SV estimates a higher fulfilment of recommendations for structural fibre than other systems. Assuming approximately appropriate diets, this indicates an overestimation of actual structural fibre supply. peNDF resulted in reasonable estimates; it reacted sensible to high contents of long-chopped grass silage in the diet. The better correlation with indicators like milk fat/protein ratio was an important advantage of peNDF over seCF in our study.

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125.

Different feeding intensities during the first four weeks of rearing in dairy calves: Part 1: Effects on performance and production from birth over the first lactation

Einfluss der Fütterungsintensität innerhalb der ersten 4 Lebenswochen auf Leistungsparameter sowie Milchleistung in der 1. Laktation

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Intensified feeding during the first weeks of life may alter the phenotypic expression for milk yield. Some recent studies compared the effects of controlled nutrient intake versus *ad libitum* feeding of calves from birth up 8 weeks of life. Increasing the nutrient intake from milk during this time resulted in increased milk yield ranging from 450 to 1,300 kg during the first lactation compared with the milk yield of restrictively fed calves during the same period (1; 2; 3). We aimed to test the effects of *ad libitum* (al) feeding of whole milk (WM) or milk replacer (MR) and of restrictive (r) feeding of MR during the first 4 weeks of life on growth performance and on milk yield in the first lactation.

Material and Methods: German Holstein calves (female: n = 29; male: n = 28) were studied from birth until d 110 of life (Trial 1). In addition, 28 heifers from Trial 1 were studied over their first lactation (Trial 2). In Trial 1, the calves were allocated directly after birth to 3 groups: MRr (n = 20, 6 L MR/calf/day), MRal (n = 17) and WMal (n = 20). All calves received colostrum from their dams until d 3 post natum (p.n.) restricted to 6 L/d for the MRr group and with al access for the MRal and WMal group. From d 4 until d 28 p.n., the calves were fed according to their group regimen. From d 28 until day 55 p.n. all calves were changed to MRr feeding and were then gradually weaned from d 56 to d 69 p.n. During the liquid feeding period all calves had access to maximally 2.5 kg concentrates/d. From d 70 until the end of Trial 1, all calves had free access to a total mixed ration. In Trial 2, dry matter intake (DMI), feed efficiency, milk yield and milk composition were recorded over 305 days of first lactation from the 28 heifers from trial 1. Body weight data were collected both in Trial 1 weekly from birth until day 110 p.n. and in Trial 2 monthly ante partum and daily over 305 days in first lactation. Data were analyzed with a mixed model of SPSS 22.

Results: Trial 1: MR and WM intakes were higher in the MRal and the WMal group when compared against the MRr group during the first 4 weeks of life ($p < 0.05$). In this period, concentrate intake was not different between the groups, but from d 28 until d 70 p.n it was 207 g/calf/day greater ($p < 0.05$) in WMal in contrast to the MRal. Average daily weight gain (ADG) was higher in MRal and WMal calves than in MRr animals ($p < 0.05$) in the first 4 weeks of life but when considering d 1 to 104 p.n., no such difference was observed. Similarly, body weight was higher ($p < 0.05$) at d 28 p.n. in MRal and WMal than in MRr calves but did not differ between the groups at 104 d of age.

Trial 2: Age at first calving (AFC) and DMI over the first 10 weeks of lactation were not different between the groups. The 305-days-milk yield and milk ingredients did also not differ between the groups, and there was no difference in body weight during the first lactation.

Conclusion: *Ad libitum* feeding over the first 4 weeks of life increased ADG and body weight in the first 4 weeks of life but the restrictively fed calves caught up and thereafter reached the same body weights as the other groups at 104 d p.n. *Ad libitum* milk feeding did not impair the concentrate intake when compared to restrictively fed calves. The intensive feeding regimen during the first 4 weeks of life had no effect of AFC, DMI or milk yield. Preweaning costs were higher for the calves fed *ad libitum.*

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126.

Impact of the first calving on mitochondrial DNA copy number and on mitochondrial enzyme activities in subcutaneous adipose tissue of dairy cows

Auswirkung der ersten Kalbung auf die Anzahl mitochondrialer DNA-Kopien und die Aktivität mitochondrialer Enzyme im subkutanen Fettgewebe von Milchkühen

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Mitochondria are the main sites of energy metabolism in mammalian cells. Their numbers as well as function depend on the energy demand and physiological state of each individual. Mitochondria possess their own genome, the mitochondrial DNA (mtDNA), and its copy numbers reflect the abundance of mitochondria within a cell. Adipose tissue (AT) is a key organ that actively participates in the adaptation to metabolic needs. In particular around calving and early lactation, high-yielding dairy cows mobilize body fat reserves due to decreased energy intake and increased energy demands for milk synthesis. In the course of pregnancy and lactation, environmental, physiological, and energetic conditions alter; therefore, we hypothesized that in AT the cellular energy production, assessed by the number of mtDNA copies/cell as well as the activity of two key enzymes participating in the mitochondrial respiratory chain, i.e., citrate synthase (CS) and cytochrome *c* oxidase (COX), is influenced by the first calving in dairy cows.

Methods: Eight pregnant German Holstein heifers ("lactating group") and eight same-aged non-pregnant and non-lactating control cows ("non-lactating group") were kept in an open barn and fed according to their requirements. Non-lactating animals received a straw-grass silage (50:50; 6.05 - 6.19 MJ NE_L/kg DM) offered *ad libitum*, and lactating heifers were fed a partial mixed ration (6.3 - 6.8 MJ NE_L/kg DM) *ad libitum* and concentrate feed (7.7 MJ NEL/kg DM) depending on the individual's milk yield. Animals had a mean body weight of 486 ± 12.2 kg and a body condition score of 3.1 ± 0.1 (5-point scale (1)). Blood samples from the jugular vein as well as subcutaneous AT from the tailhead region were collected in week (wk) 7 of pregnancy and 3 wk postpartum (p.p.). After sampling, AT biopsies were immediately snap-frozen in liquid nitrogen. Genomic DNA was isolated and AT extracts were prepared after homogenization. The relative mtDNA copy number/cell was quantified by multiplex qPCR and calculated as described previously (2). The activities of CS and COX were determined spectrophotometrically in AT extracts using commercially available kits. Concentrations of non-esterified fatty acids (NEFA) were analyzed in serum samples by an automatic clinical chemistry analyzer. Data (means ± SEM) were analyzed by linear mixed models; the associations between variables were assessed by the Spearman correlation (SPSS).

Results: In the lactating group, the number of mtDNA copies/cell decreased by half from pregnancy to early lactation ($P < 0.001$), whereas in the non-lactating group the number of mtDNA copies/cell remained unaffected. Moreover, in early lactation, the number of mtDNA copies/cell was 1.8-fold higher ($P< 0.001$) in AT from non-lactating compared to lactating animals. From pregnancy to early lactation, activities of CS and COX neither changed in the lactating nor in the non-lactating group. However, in early lactation, activities of both enzymes were higher (CS: 1.7-fold, $P = 0.039$; COX: 2.3-fold, $P = 0.032$) in non-lactating animals. Over all data, positive correlations were observed between CS and COX activity ($\rho = 0.499$; $P = 0.003$) and CS activity and mtDNA copies/cell ($\rho = 0.364$; $P = 0.034$). Furthermore, the number of mtDNA copies/cell tended to be related to COX activity ($\rho = 0.297$; $P = 0.088$) and was negatively correlated with NEFA concentrations ($\rho = -0.366$; $P = 0.033$).

Conclusions: High NEFA concentrations in early lactation of lactating cows indicate increased lipolysis in AT. Decreased numbers of mtDNA copies/cell in AT, suggest that the cellular energy production by mitochondria fails to meet the increased energy demands in early lactation. For the negative correlation between NEFA and mtDNA, it remains open as to whether decreased mtDNA copy numbers resulted directly from increased NEFA concentrations (3) or were indirectly related. Due to the fact that mitochondrial activity, assessed by CS and COX activity, remained unaffected from pregnancy to early lactation, further studies are necessary to describe the regulation of mitochondrial function and biogenesis in bovine AT.

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127.

Energy metabolism determined by indirect calorimetry during the first week of life in calves fed either colostrum or formula

Energiestoffwechsel gemessen mittels indirekter Kalorimetrie in der ersten Lebenswoche bei Kälbern mit Kolostrum- oder Formulafütterung

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After birth, the calf's energy supply changes from parenteral carbohydrate supply, mainly glucose, by the placenta to oral fat and carbohydrate supply, mainly triglycerides and lactose, by colostrum. In addition to nutrients colostrum provides a plethora of bioactive substances like immunoglobulins, cytokines, hormones and growth factors. The latter ones promote the development of the gastrointestinal tract, which is the first site of nutrient absorbance. So, maturation of the gastrointestinal tract and especially of the small intestine improves total energy supply. Energy expenditure (EE) can be calculated from data gained by indirect calorimetry. Such calorimetric data, which further clarify substrate utilisation immediately after birth, are rare for neonatal calves. Thus, the purpose of this study was to gain data on substrate utilisation and EE in neonatal calves during the first week of life when fed either colostrum or a milk-based formula. We hypothesized that substrate utilisation and EE change with age and are influenced by the diet fed immediately after birth.

Methods: German Holstein bull calves (n = 13) were separated from their dams immediately after birth before first feed intake. For the first two days of life, calves were fed either pooled colostrum (n = 7) or a milk-based formula (n = 6) with a similar nutrient composition as colostrum but almost without bioactive substances based on milk components (casein, lactalbumin, lactose, coconut oil) (1). From d 3 until the end of the study calves received milk replacer (150 g powder per 1 l water). Amounts fed were 10% of birth weight on day 1 and 12% from d 2 on. Calves were fed twice daily (7:00 and 17:00) by nipple bottle or bucket. On the first two days refused amounts of feed were tube-fed to ensure complete feed intake. On d 2 and 7 of life, calves were placed in straw-bedded fully air-conditioned respiration chambers for indirect calorimetric measurements. After gas exchange equilibration time CO_2 production and O_2 consumption were measured for 21 hours in 6 min intervals (16:00 to 13:00) to calculate EE, fat and carbohydrate oxidation as well as respiratory quotient. In addition, physical activity in the chamber was measured by an infrared sensor. Data were analysed by mixed model of SAS 9.3 with group, day, hour and their interactions as fixed effects.

Results: Calculated EE, fat and carbohydrate oxidation and respiratory quotient differed significantly ($P < 0.05$) for day, hour, day x hour interaction and group x day x hour interaction. Comparing d 2 and 7 revealed an increasing carbohydrate oxidation and respiratory quotient and a decreasing fat oxidation and EE with age. Variations in diurnal rhythms were more pronounced on d 2 than on d 7, especially for EE and fat and carbohydrate oxidation. Energy expenditure and carbohydrate oxidation during the first hours measured on d 2 were numerically higher in formula- than in colostrum-fed calves. The activity in the chamber was higher in colostrum- than in formula-fed calves and decreased during first week of life (group, day $P < 0.05$).

Conclusions: Evaluating our preliminary data indicate that different initial diets for two days may affect energy metabolism on d 2, but have no long-lasting effects on substrate utilisation and EE, whereas both are influenced by ontogenic maturation. Energy expenditure at birth is higher than after the first week of age when adaptation to extra-uterine life proceeds and metabolic processes are more mature. Furthermore, diurnal rhythms of EE and substrate oxidation become more apparent with increasing age. Reduced activity on d 7, when compared to d 2, may be due to habituation to the respiratory chambers and higher activity of colostrum-fed calves is probably a sign of elevated fitness of these calves.

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128.

Effects of conjugated linoleic acids (CLA) and Vitamin E on fat depot mass changes in periparturient dairy cows

Effekte von konjugierten Linolsäuren und Vitamin E auf Fettdepotmasse- Veränderungen bei Milchkühen im peripartalen Zeitraum

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Overconditioned dairy cows are subjected to intensive fat depot mobilization and are predisposed to subclincal ketosis (sk) after calving. Therefore, the present study was conducted to examine the anti-lipolytic effects of CLA and Vitamin E (VE) on fat depots in the periparturient period.

Methods: Thirty-one pluriparous Holstein cows were fed according to an animal model to induce sk (1). Cows received 42 days (d) *ante partum* (a.p.) a diet consisting of 60% concentrate (on dry matter basis). After parturition the concentrate proportion in the diet was 30% and increased within 21 d up to a level of 50% concentrate. Cows were assigned to four treatments in a 2 x 2 factorial experimental design with the factors CLA and VE according to their body condition score 42 d a.p. Cows of the control group (CON, n = 8) received no *trans*10,*cis*12 (*t*10,*c*12) CLA + 500 IU VE per d, the CLA group (CLA, n = 8) 8 g *t*10,*c*12 CLA + 500 IU VE per d, the VE group (n = 8) no *t*10,*c*12 CLA + 3000 IU VE per d and the CLA/VE group (n = 7) 8 g *t*10,*c*12 CLA + 3000 IU VE per d. Cows of the CON group and VE group received a control fat preparation with no CLA. For quantification of the fat tissues ultrasound measurements were conducted at 42 d a.p., 7, 28, and 70 d *post partum* (p.p.). The subcutaneous (SCAT), retroperitoneal (RPAT), omental (OMAT) and mesenteric (MAT) fat depot masses were estimated using regression equations (2). Daily mobilization/ accretion of a fat depot was calculated as follows: Fat depot mass at the end minus the fat depot mass at the start of the observed period. This difference was divided by the days of the period to obtain the daily mobilization/accretion of the respective fat depot. The periods were defined as dry period + 7 d p.p. (DP+7pp; 42 d a.p. to 7 d p.p.), fresh cow period (FC; 7 d p.p. to 28 d p.p.) and early lactation period (EL; 28 d p.p. to 70 d p.p.). Data were analysed using the GLM procedure of SAS 9.4.

Results: The sum of fat depot change was different between all viewed periods ($P \leq 0.001$) (Figure 1). In the DP+7pp period the highest accretion was observed for the SCAT. The change of SCAT of the DP+7pp period was different compared to the FC ($P < 0.001$) and EL period ($P < 0.001$). While for the FC and EL period no difference ($P = 0.122$) of SCAT mobilization was observed (Figure 2). In no period a significant effect of treatment on fat depot changes was detectable.

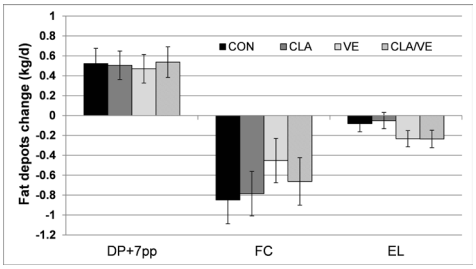

Figure 1: Fat depots changes (sum of SCAT, RPAT, OMAT and MAT; positive values = accretion; negative values = mobilization) during the different periods (LS-means ± SE)

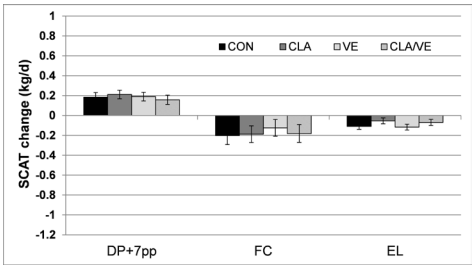

Figure 2: SCAT changes (positive values = accretion; negative values = mobilization) during the different periods (LS-mean ± SE)

Conclusion: In the present study only longitudinal time effects between the viewed periods on fat depot changes were observed. CLA and VE treatment showed no influence on fat depot changes.

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129.

Effects of intensive milk feeding and butyrate on metabolic and endocrine traits in blood and growth performance in German Holstein calves

Einfluss einer intensiven Milchfütterung und von Buttersäure auf metabolische und endokrine Blutparameter und das Wachstum bei Kälbern der Rasse Deutsche Holstein

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Contrary to conventional feeding strategies with limited supply of milk previous studies have shown that calves are able to digest and metabolize much greater amounts of milk when feeding *ad libitum* (1). On the other hand butyric acid is known to stimulate intestinal growth and development in milk-fed calves (2). Combining these strategies of intensive milk feeding and butyrate supplementation, we hypothesised in the present study that this combination accelerate postnatal growth performance and anabolic metabolism in calves.

Methods: 64 German Holstein calves (♀ n=32, ♂ n=32) were born between June 2014 and March 2015 and studied from birth until d 81 (±2) post natum. All calves received first colostrum (2.5 kg from their dams within ca. 2 h after birth. Subsequent colostrum meals (two colostrum meals/d until d 3) and milk replacer (MR; 125 g powder/l) were either offered in amounts of 6 l/d (Res; n=32) or *ad libitum* (Adlib; max. 25 l/d, n=32) for 8 wk. In both feeding regimes, half of the calves (n=16/group) were fed MR with 0.33% Ca-/Na-butyrate (ResB+; AdlibB+) or same MR with no butyrate supplement (ResB-; AdlibB-). Milk intake for all calves was stepped down to 2 l/d from wk 8 to wk 10, and 2 kg MR were offered until the end of the study. Concentrate, hay and water were freely available. The calves were individually housed in calf hutches for the first 10 d (±3) and afterwards in an open straw-bedded stable with an automatic feeding system. On d 1 (before colostrum intake), 2, 3 and 7, then weekly until wk 11 of life, blood samples were taken to measure plasma concentrations of several metabolites (glucose, β-hydroxybutyrate [BHBA], non-esterified fatty acids [NEFA], triglyceride, cholesterol, total bilirubin, lactate, albumin, total protein, urea) as well as insulin and glucagon. Feed intake was measured daily, and calves were weighed after birth and then weekly until the end of the experiment. Performance and blood data were analysed by the Mixed Model of SAS with feeding regime, butyrate supplementation, time, and respective interactions as fixed effects.

Results: Birth weight (43.6 ± 4.4 kg; mean ± SD) and first colostrum intake (2.5 ± 0.09 kg) were similar in all groups. Intake of MR was greater ($P < 0.001$; max. MR intake: 13.5 ± 0.5 kg/d in wk 8 and 9), concentrate intake was lower ($P < 0.001$), and body weight increased much more ($P < 0.001$) in Adlib than in Res. Plasma concentrations of glucose and insulin from d 7 to d 63 as well as the glucagon and urea rise until d 3 after birth were greater ($P < 0.001$) in Adlib than Res, but the BHBA rise occurred earlier ($P < 0.001$) in Res than in Adlib. Total bilirubin concentrations were highest ($P < 0.001$) on d 2 in all groups, but the decrease thereafter was delayed in ResB+. Plasma lactate, NEFA, total protein, triglyceride and cholesterol concentrations changed with time, but showed no marked group effects.

Conclusions: Intensive milk feeding resulted in enhanced body growth and metabolic and endocrine changes, indicating greater stimulation of anabolic metabolism in Adlib than in Res calves. Butyrate supplementation did not improve growth performance and the effects on metabolic and endocrine traits by butyrate were weak, independent of milk feeding regime. The increase in concentrate intake was delayed in Adlib, but Adlib calves ended with the same level of concentrate intake after step down from liquid feed, which indicated no impairment on rumen development in Adlib calves.

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130.

Interrelation between activation and proliferative response of blood leukocytes as well as the energy status in high-yielding dairy cows around calving

Zusammenhang zwischen Aktivierung und Proliferation von Leukozyten aus dem Blut sowie dem Energiestatus von hochleistenden Milchkühen im peripartalen Zeitraum

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In the peripartum state, all mammals encounter a breakdown of immunity. During the specific energy-demanding time of late pregnancy and early lactation the incidence of parasite load and inflammatory diseases often rise. Using *in vitro* techniques, blood leukocytes were shown to display decreased DNA synthesis (proliferation) in response to mitogens in this period. The phase of the cell cycle preceding DNA synthetic phase, the so-called leukocyte activation, may already be impaired by the limited energy/nutrient availability. In the first weeks following calving the energy deficiency is particularly severe due to the high milk production. In the present study, it was tested whether the proliferation of peripheral blood mononuclear cells (PBMC) is related to PBMC activation and the animal's energy status as assessed by feed intake, feed efficiency and mobilization of body reserves.

Methods: Eleven multiparous Holstein cows receiving a total mixed ration were included in this study. Their feed intake, milk yield, body condition score (BCS) and backfat thickness (BFT) were recorded 2 weeks before and 2 and 12 weeks after calving. Activation and proliferation of PBMC were studied 24 and 72 h after *in vitro* stimulation with phytohaemagglutine (4 µg/ml) by oxygen consumption measurement (1) and the MTT assay, respectively. Activation and proliferation responses were calculated as the difference of stimulated and the non-stimulated control PBMC divided by the non-stimulated control PBMC. Using the data from week 2 postpartum, cows were grouped by the extent of the proliferative response into high- (HP; 6 cows; 1.5 ± 0.3 dimensionless unit, non-stimulated control=0) and low-proliferators (LP; 5 cows; 0.6 ± 0.2). Data were evaluated by a two-way repeated measurement ANOVA with Holm-Šídák adjustment. Group, week and their interaction were considered as effects.

Results: High- and low-proliferators did not differ in intake (per metabolic body weight), but intake increased with time in HP. There were no group differences in feed efficiency (energy-corrected milk yield per intake), BCS and BFT. The latter two decreased with time. In PBMC proliferation, there was a group x week interaction, with group differences at weeks -2 (HP<LP) and +2 (HP>LP) relative to calving. In HP there was an effect of time (week -2<+2>+12). Activation of PBMC was not affected by group. The analysis of blood metabolites, cell cycle analysis by flow cytometry and qRT-PCR are underway.

Conclusion: Different from what was expected, the PBMC proliferation in week 2 after calving was not related to PBMC activation and the animal's feed intake, feed efficiency and body reserve mobilization. Interestingly, LP cows (low-proliferators in week +2) displayed a high PBMC proliferation in week -2. It could be speculated that the approaching calving event challenged PBMC in week -2 to respond intensively to a mitogen, whereas the preceding parturition imprinted PBMC in week +2 in a way to allow only a low response.The first results on energy metabolism indicate that the amount of feed energy partitioned towards milk production do not explain the observed differences in PBMC proliferation.

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131.

Association between body fat mobilization in early lactation and methane emission from Holstein dairy cows

Zusammenhang zwischen Körperfettmobilisierung in der Frühlaktation und der Methanproduktion bei Holstein Kühen

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The mammary gland of dairy cows uses large amounts of acetate for the *de novo* synthesis of milk fat. In early lactation energy requirements for maintenance and milk production exceed the energy supply from ingested feed. As a consequence, high-yielding dairy cows enter a negative energy balance and mobilize their body fat reserves. Mobilized long-chain fatty acids are intensively used for milk fat synthesis resulting in diminished utilization of acetate as precursor for milk fat *de novo* synthesis. Acetate is produced during ruminal fermentation of ingested feed and its production is stoichiometrically linked with the production of hydrogen. Methanogenic archaea utilize the released hydrogen to produce methane (CH_4). Thus, the amount of CH_4 produced is directly correlated with ruminal acetate production. We hypothesized that decreased acetate utilization by the host would negatively affect ruminal acetate and thus CH_4 production.

Methods: Twenty pregnant German Holstein heifers were monitored from week 4 prior to parturition until week 42 in lactation. Body weight and back-fat thickness were measured every two weeks. During lactation, milk yield was measured daily whereas milk constitutes were analyzed weekly. Feed intake was measured daily and dry matter and neutral detergent fibre (NDF) contents were analyzed once a week. In week -4, +5, +13 and +42 relative to parturition, animals were sampled for jugular blood plasma and rumen fluid and transferred into closed circuit respiration chambers to measure CH_4 production for 24 h. Based on the plasma non-esterified fatty acid (NEFA) concentration determined in week +5, animals were grouped into ten high mobilizing (HM; NEFA > 580 µmol/L) and ten low mobilizing (LM; NEFA < 580 µmol/L) cows.

Results: While DMI, milk yield, rumen fluid pH and concentrations of ruminal acetate, propionate and butyrate did not differ between groups, methane yield corrected for DMI tended to be lower in HM cows in early lactation ($P = 0.1$). Furthermore, we found a negative regression between NEFA concentration and CH_4/DMI or CH_4/NDF ($P = 0.002$ and $P = 0.005$, respectively), and an inverse relationship between NEFA and plasma acetate concentrations ($P = 0.053$).

Conclusions: Our data shows for the first time that circulating long-chain fatty acid concentrations are indirectly related to CH_4 production and that this effect is not based on DMI. However, in contrast to our hypothesis, ruminal acetate concentration was not affected by fat mobilization, indicating that the mechanism for the CH_4 reducing effect remains to be investigated.

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132.

Heat stress effects hepatic gluconeogenesis of late-gestation dairy cows

Hitzestress beeinflusst die die hepatische Gluconeogenese in der Spätträchtigkeit der Milchkuh

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The two major stressors adversely affecting the performance of dairy cows are the metabolic stress during the transition period from late pregnancy to early lactation, and environmental heat stress. The combination of these two stressors has severe negative consequences on fetal growth, milk production and metabolic health. The liver of late-pregnant cows has a central role in suppling glucose to the growing fetus. However, the molecular mechanisms underlying the adaptation of hepatic gluconeogenesis and energy production to ambient heat have not been evaluated yet. Therefore, the objective of this study was to analyze the effect of heat stress on glucose and mitochondrial metabolism in the liver of cows.

Methods: Twelve German Holstein dairy cows were grouped to heat-stressed (HS) and pair-feeding (PF). Twenty one days before parturition, both groups were exposed to the same climate condition (15°C, relative humidity (RH) = 63.3%, temperature humidity index (THI) = 59) with *ad libitum* feeding for one week (P1). On the following transition day, the air temperature was gradually increased to permanent 28°C (RH = 52%, THI = 76) for heat-stress and maintained at 15°C (RH = 69%, THI = 60) for pair-fed animals and groups were kept under these conditions for 6 further days (P2). Feed intake was recorded daily. Liver biopsies were taken in the morning on transition day (reflecting P1) and on day 6 of period 2 after heat-stress. Blood samples were taken daily in P2 for lactate analysis using ABX Pentra 400. Liver tissues were used for mRNA expression analysis using RT-qPCR (pyruvate carboxylase (*PC),* phosphoenolpyruvate carboxykinase 1 and 2 *(PCK1& PCK2),* lactate dehydrogenase A *(LDHA),* citrate synthase *(CS),* NADH dehydrogenase subunit 2 *(ND2),* ATP synthase *(Atp5b)*). Differences between periods P1 and P2 of the same group were analyzed using the Wilcoxon signed rank sum test included in the UNIVARIATE procedure of SAS. Repeated measures data were analyzed for the effects of group, day, and their interaction during period 2 as a completely randomized design using PROC MIXED model of SAS (Version 9.4).

Results: Dry matter intake declined by 37-40 % in both cow groups. In cows kept under HS conditions, *PC* and cytosolic *PCK1* mRNA abundance increased (3.2-3.4-fold, PPCK2 mRNA remained unaffected. In PF animals, *PC* mRNA abundance tended to increase (1.9-fold, PPCK1 mRNA abundance decreased from P1 to P2 (1.2-fold, PLDHA mRNA abundance in P2 relative to P1 (1.8-fold, P CS, *ND2* and *Atp5b* mRNA remained unaltered.

Conclusion: These results indicate increased utilization of cytosolic oxaloacetate and pyruvate for gluconeogenesis in HS compared to PF animals. This might refer to increased utilization of alanine for gluconeogenesis under heat stress conditions. Furthermore, heat stress seems not to compromise utilization of lactate for gluconeogenesis despite higher plasma lactate concentrations in HS cows at the end of P2. Thus, utilization of substrates for gluconeogenesis shifts from propionate to pyruvate and cytosolic oxaloacetate, while mitochondrial energy metabolism is not affected by this substrate shift.

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133.

Pure flavonoids and their influence on ruminal methanogenesis and fermentation as determined *in vitro*

Reine Flavonoide und deren Einfluss auf die ruminale Methanbildung und Fermentation im in vitro-Versuch

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Some phytogenic compounds such as polyphenols have been shown to have a potential to mitigate methane emission from ruminants (1). In the present study polyphenols belonging to the group of flavonoids, with known health-promoting effects in humans, were examined in a short-term *in vitro* experiment. The aim was to evaluate their methane-mitigating potential and their effects on ruminal fermentation when added as pure compounds to a basal diet.

Methods: The Hohenheim Gas Test method was used to compare five flavonoids, namely epicatechin, luteolin-7-glucosid, quercetin, isoquercitrin and tannic acid in four experimental runs each. Thereby, 200 mg dry matter of ryegrass hay was supplemented with 0, 0.1, 1 or 10 mg of the five compounds and incubated in 30 ml ruminal fluid-buffer solution (1:3) for 24 h at 39 °C. Ruminal fluid was collected before morning feeding from a rumen-fistulated dairy cow. The not supplemented ryegrass hay and the known methane-mitigating compound tannic acid acted as controls. After the incubation was stopped, incubation fluid was analysed for pH, ammonia concentration, short chain fatty acids (SCFA), as well as protozoal and bacterial counts. Fermentation gas production and CH_4 concentration was determined as well. Statistical analysis was done using the MIXED procedure of SAS considering dietary treatment and run as factors.

Results: The 10-mg additions of luteolin-7-glucosid and quercetin decreased ($P < 0.05$) absolute CH_4 formation similar to the 10-mg dosage of tannic acid compared to the not supplemented control. However, all 10-mg dosages decreased ($P < 0.05$) CH_4 proportion in fermentation gas compared to the control. The levels of decline were 12.3, 17.4, 15.0 with 13.5% with epicatechin, luteolin, quercetin and isoquercitrin, respectively, and were thus almost as efficient as tannic acid (-17.0%). Fermentation gas formation was not affected by any of the flavonoid supplementations. The supplementation with any flavonoid also had no significant effect on either bacterial or protozoal counts. While total SCFA production was higher with epicatechin and luteolin-7-glucosid ($P < 0.05$) when compared to the not supplemented control, the SCFA profile did not differ between the treatments. A tendency for a decline in incubation fluid ammonia concentration (-13%; $P = 0.06$) was found with the 10-mg dosage of luteolin-7-glucosid, whereas this effect was significant (-17%) for the 10-mg dosage of tannic acid.

Conclusion: Flavonoids, and thereof especially tannic acid and luteolin-7-glucosid, have the potential to mitigate methane formation in ruminal fermentation *in vitro*. In addition, according to the high SCFA production, luteolin-7-glucosid seems to increase nutrient degradation *in vitro*. The decrease in incubation fluid ammonia concentration with tannic acid and, numerically, with luteolin-7-glucosid indicates a potential to improve the ruminant's N-metabolism as well. Long term *in vitro* as well as *in vivo* studies need to be performed to confirm these effects.

(1) DIJKSTRA, J, OENEMA, O and BANNINK, A (2011): Current Opinion in Environmental Sustainability 3, 414-422

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134.

Effects of feeding diets differing in starch and fibre content with or without linseed supplementation on methane emission and milk fatty acids in dairy cows - first results

Effekte von verschiedenen Futterrationen, die sich im Stärke- und Fasergehalt unterscheiden, mit und ohne Leinsamensupplementierung, auf die Methanemission und die Milchfettsäuren bei Kühen - Erste Ergebnisse

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Methane produced by ruminants contributes to global warming. Preliminary data suggest that genetic selection to reduce emissions is possible. However, breeding programs require large datasets of individual animal measurements which is not possible to generate using classical gas exchange measurements in respiration chambers. In addition, it is known that dry matter intake (DMI) and feed composition strongly affect methane emission. Currently, some easier to measure proxies for individual methane emission are under investigation, and one of them is milk fatty acid concentrations. The purpose of the study is to delineate the relationship between individual methane output and milk fatty acid profile using diverse feed composition. The aim is to deduce an improved milk fatty acid marker to predict individual methane emission.

Material and Methods: Twenty lactating (106 ± 28 lact. day, 580 ± 57 kg BW), half-sib German Holstein cows were fed ad libitum 4 diets: starch-based **(S)**, S + linseed **(SL)**, rich in fiber **(RF)**, RF + linseed **(RFL)**. Diets S and RF contained grass and corn silage at 15.4% and 62.5%, and 48.6% and 25.8%, respectively. In wk 1, cows were switched from standard TMR to one of the 4 diets, and were continued on this diet until wk 6. Thereafter, from wk 7 to 12, diet was changed from RF to RFL or vice versa and from S to SL or vice versa. Methane emissions were recorded in wk 4 and 10 for 48 h each, using open circuit respiration chambers. During respiration measurement a milk aliquot (evening and morning milking) was collected and analysed by near-infrared spectroscopy. So far, data from 12 cows were analyzed using PROC MIXED of SAS.

Results: Preliminary findings indicate higher DMI with the S diets and similar CH_4 emission per kg DMI with the RF and the S diets, whereas a methane lowering effect of linseed supplementation was found (Table 1). Methane output per day ranged from 337 to 708 L/d. Linseed decreased saturated fatty acid (SFA) and increased unsaturated fatty acids (USFA) in milk fat.

Table 1 Effects of 4 different diets on methane and performance parameters (n=6/diet)

	RF	RFL	S	SL	SE	P-value
DMI (kg)	14.8^{c}	16.4^{ab}	18.0^{a}	17.7^{a}	0.83	0.011
ECM (kg)	19.8^{c}	20.6^{c}	28.1^{a}	26.3^{b}	1.35	0.001
CH_4 L/kg DMI	32.3^{a}	26.9^{b}	31.1^{a}	26.7^{b}	1.39	0.001
Milk						
Fat (g/L)	45.8	45.0	40.1	38.2	2.6	0.11
SFA (%)	70.8^{a}	60.9^{b}	71.2^{a}	60.6^{b}	1.1	0.001
USFA (%)	29.2^{b}	39.2^{a}	28.8^{b}	39.4^{a}	1.1	0.001

Conclusion: Our preliminary data shows that the experimental diets cause a wide variation of methane output associated with significant changes of the SFA/USFA ratio. This suggests that the selected experimental diets are suitable to construct a regression equation to predict individual methane emission from milk fatty acid profile.

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135.

Effects on the acute phase reaction (APR) in lipopolysaccharide (LPS)-stimulated pigs pre-exposed with deoxynivalenol (DON)

Einfluss auf die Akute-Phase-Reaktion in Lipopolysaccharid-stimmulierten, zuvor Deoxynivalenol-exponierten, Schweinen

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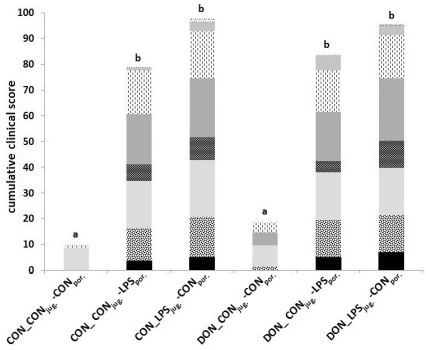
Pigs are often exposed to DON, a *Fusarium* mycotoxin, and LPS, an endotoxin of gram-negative bacteria, in their environment. Both toxins mainly enter the organism via the gastrointestinal tract and are known for their immune modulatory, partly synergistic effects with respect to the APR [1], but information about their interaction with respect to the porcine immune response is limited. The aim of our study was to determine the effects of DON and LPS on the APR in pigs in more detail.

Material & Methods: Barrows (German landrace, n=44) were fed restrictively (2x700g/d) either with a control- (53.3% barley, 15% maize) or a naturally contaminated DON-diet (53.3% barley, 15% mycotoxin maize, 4.59mg DON/kg feed) for four weeks. They were surgically equipped with an intraabdominal temperature logger and catheters at different locations to enable pre- (*V. portae hepatis*, *V. lienalis*) or post-hepatic (*V. jugularis interna* and *externa*) applications and simultaneous blood sampling. This resulted in six experimental groups, whereby the first abbreviation refers to the diet and the other two indicate the jugular and portal infusion: CON_CON$_{jug}$-CON$_{por}$, CON_CON$_{jug}$-LPS$_{por}$, CON_LPS$_{jug}$-CON$_{por}$, DON_CON$_{jug}$-CON$_{por}$, DON_CON$_{jug}$-LPS$_{por}$, DON_LPS$_{jug}$-CON$_{por}$. Animals were infused with either 0.9% NaCl or LPS (7.5µg/kg BW) for 60min and blood samples were taken every 15 to 30min for leukocyte counts and TNF-alpha, starting 30min before until 180min after start of infusion. Concurrently clinical signs were scored (table in Fig. 1) every 15min.

Results: All LPS-infused animals showed a significant increase in cumulative clinical score (ppost infusionem (*p.i.*) with tremor and cyanosis followed by hyperaemic conjunctivae and injected episcleral vessels. Also, an increase in body core temperature (~1.5°C) was observed in all LPS groups (pjug.-CON$_{por}$. resulted in a lower temperature-rise (by ~0.5°C) than CON_LPS$_{jug}$.CON$_{por}$. (p=0.08). Additionally, LPS induced a severe leukopenia in all LPS-infused pigs (pp.i. wherein DON-fed and jugular-infused animals showed significantly lower levels already 15min earlier than their counterparts (p3/µl) at -30min compared to CON-fed group (p=0.04). TNF-alpha rapidly peaked with ~140ng/ml at 60min *p.i.*, followed by a gradual decrease to almost base levels at 180min *p.i.* in LPS stimulated pigs, irrespective of oral DON exposure.

Conclusion: Based on our data and data from literature we suggest that chronic DON-feeding, in combination with a subsequent LPS-stimulus, on the one hand overstrains the functional capacity of the liver as indicated by the earlier and stronger leukopenia and on the other hand accelerates the innate and adaptive immune response as evidenced by lower levels of hyperthermia.

1. Pestka, J.J., et al., Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. Toxicol Lett, 2004. 153(1): p. 61-73.



Score	0	1	2	3	4
nystagmus	-	nystagmus present			
teeth gnashing	-	teeth gnashing present			
dermographism	-	skin coloring pattern present			
vomiting, retching	-	smacking, retching, foam-forming	vomiting of slime	vomiting of feed/digesta	vomiting of slime and feed/ digesta
hyperaemic conjunctivae	physiological	(rose) red	red		
injected episcleral vessels	-	slightly injected	medium injected	highly injected	
respiratory difficulties	-	low laboured breathing	medium laboured breathing	severe laboured breathing	open-mouth breathing
respiratory rate	10-20/min	21-40/min	41-60/min	61-80/min	> 80/min
cyanosis	-	low cyanosis	medium cyanosis	severe cyanosis	
tremor	-	low shivering	medium shivering	severe shivering	spasms

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136.

Effects of a lipopolysaccharide (LPS) stimulus in pigs chronically exposed to dietary deoxynivalenol (DON): Metabolic and haematological consequences

Metabolische und hämatologische Auswirkungen eines systemischen E.coli Lipopolysaccharid (LPS) Stimulus bei Schweinen nach chronischer Deoxynivalenol (DON) Fütterung

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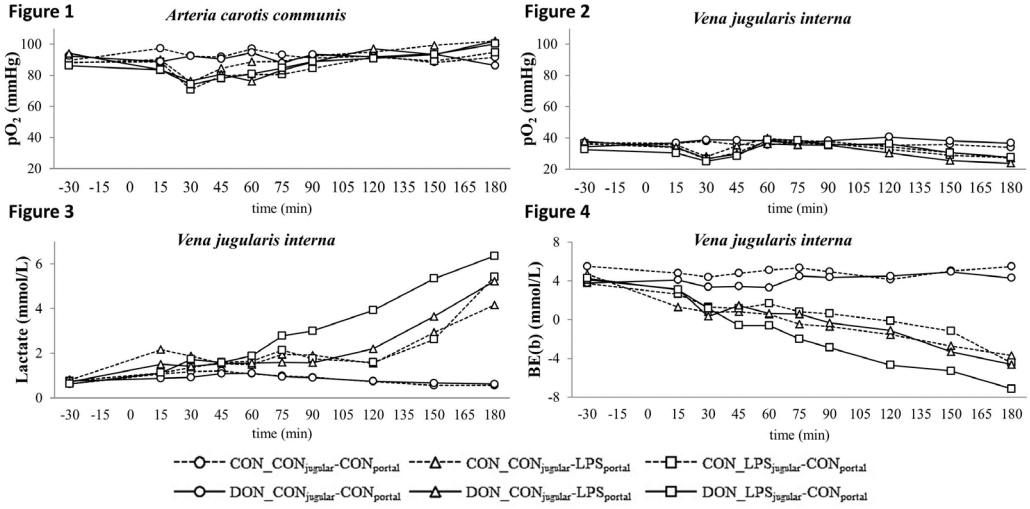
The Fusarium toxin deoxynivalenol (DON) is in temperate climate zones one of the most important mycotoxins in cereals. Especially pigs are very sensitive to the impact of DON. Investigating a potential modulatory effect of DON to a subsequent immunological stimulus in pigs was one of the main objectives of the present project.

Methods: In our trial a total of 41 barrows were examined of which 19 were chronically exposed to a DON contaminated maize-based diet (4.59 mg DON/kg feed) and 22 to an uncontaminated control feed. This resulted in two feeding groups (CON or DON). All pigs were equipped with catheters at different locations to enable pre- (*V. portae hepatis*, *V. lienalis*) or post-hepatic (*V. jugularis interna* and *externa*) applications (LPS, 7.5 µg/kg BW; control, 0.9% NaCl) and simultaneous blood sampling. An additional catheter was placed into the carotid artery for sampling only. Each feeding group was divided into three infusion groups ($CON_{jugular}$-CON_{portal}, $CON_{jugular}$-LPS_{portal}, $LPS_{jugular}$-CON_{portal}). Frequent blood samples were taken from the different catheters before, during and after infusion (−30, +15, +30, +45, +60, +75, +90, +120, +150 and +180 min). Subsequently analyses for blood gases, electrolytes, pH, lactate (GEM4000, Werfen) and red hemogram (Celltac, Baumann Medical AG) were performed. Data were evaluated by PROC MIXED in SAS with group, catheter and time as main factors as well as their interactions.

Results: Erythrocytes, hematocrit, hemoglobin and electrolytes were not affected by DON and LPS. In LPS infused groups oxygen partial pressure (pO_2) decreased overall in venous blood until 180 min ($p < 0.05$) whereas pO_2 was reduced only at 30, 45 and 60 min in arterial blood ($p < 0.05$) (figure 1, 2). DON had no effect on pO_2 and pCO_2. Irrespective of catheter localization pH decreased ($p < 0.01$) and lactate increased ($p < 0.01$) in LPS-groups (figure 3), indicating an emerging lactic acidosis. Base excess blood (BE(b)) decreased while arterial pO_2 normalized after 90 min (figure 4, 1), indicating a metabolic acidosis. All LPS-infused groups reached maximum lactate levels at 180 min (figure 3). On lactic acidosis DON had a significant amplifying effect at post-hepatic infusion-site (DON_$LPS_{jugular}$-CON_{portal}) at 120 and 150 min ($p \leq 0.01$).

Conclusions: Chronic DON-feeding alters the porcine pathophysiological response to a subsequent LPS stimulus dependent on infusion site. In case of post-hepatic LPS-treatment the lactic acidosis is amplified by DON. This underlines the DON-priming effect and the role of the liver as detoxifying organ.



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137.

Performance and fatty acid profile in milk and digesta of dairy cows fed a total mixed ration with reduced essential fatty acid content

Leistung und Fettsäureprofil in Milch und Digesta bei Milchkühen mit reduziertem Gehalt an essentiellen Fettsäuren in der Ration

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Common diets in dairy production are based on corn and grass silage, and provide less fresh grass than pasture based systems that supply high amounts of essential fatty acids (EFA), especially a-linolenic acid (ALA). Therefore, all the more corn-based diets may deliver insufficient EFA like ALA. In a first attempt to investigate the importance of EFA in dairy production, we created a total mixed ration (TMR) with low fat and particularly low ALA content. This diet may result in less rumen production of conjugated linoleic acid (CLA) and trans-fatty acids leading to reduced tissue CLA. To verify this assumption, we investigated the fatty acid profile in duodenal ingesta and in milk. In addition, performance data were analysed to test our hypothesis that a low fat-diet with negligible ALA supply may affect milk performance.

Methods: Five duodenal fistulated lactating cows (57 DIM ± 12 d at start of the study) were investigated for 24 weeks after changing from a grass/corn silage based (GS) to a corn silage based TMR (CS). Components of CS were corn silage, straw, soybean meal, dried sugar beet pulp and rye grain. Diets were isoenergetic (6.8 MJ NEL/kg of dry matter (DM)) and isonitrogenous (crude protein 155 g/kg DM), but crude fat content was lower in CS than in GS (30.99 versus 22.38 g/kg DM for GS and CS). Content of linoleic acid (LA) was quite similar (11.7 and 10.8 g/kg DM in GS and CS), but ALA content was much lower in CS than GS diet (6.2 and 1.0 g/kg in GS and CS). DM intake (DMI), milk yield and milk composition were measured weekly. Fatty acid (FA) composition in milk was measured in week -1, 2, 4, 6, 8, 16, 20, 24, and in duodenal ingesta in week -1, 6, 8, 16, 24. Data were analysed by mixed model of SAS with time as fixed effect.

Results: DMI and energy intake increased, but total fat intake decreased with CS diet ($P<0.001$). Intake of LA increased with time but ALA intake dropped down with the CS diet and remained on a low level until the end of the study ($P<0.001$). As expected, milk yield, energy-corrected milk and fat and protein yield declined with time ($P<0.001$), but protein and fat content in milk increased at the same time ($P<0.01$). Concentration of ALA in milk and relative amount of ALA in milk fat decreased ($P<0.001$), whereas relative amount of LA in milk fat increased ($P<0.001$), and LA concentration in milk did not change during CS feeding. Concentration of *c9*, *t11* CLA and vaccenic acid in milk and milk fat decreased ($P<0.001$) with CS feeding. In duodenal ingesta, relative and absolute mass of ALA was reduced with CS feeding ($P<0.001$).

Conclusions: Data available so far support our hypothesis that the CS diet reduces ALA content in milk and results in very low ALA supply to dairy cows. Further investigations of FA concentrations in different lipid fractions of blood plasma and in membranes of red blood cells will provide more insight into fatty acid status of dairy cows fed corn silage based TMR, but not grass or grass silage.

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138.

Influence of partial replacement of fat by distilled palm kernel fatty acids (PKFAD) in a complete diet on the lauric acid content in the gastrointestinal tract of broiler chicken

Einfluss eines partiellen Austausches des Fettes im Mischfutter durch destillierte Palmkernfettsäuren auf den Laurinsäuregehalt im Gastrointestinaltrakt von Broilern

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PKFAD are characterized by a high concentration of lauric acid [1]. For that reason a partial substitution of fat in the diet by PKFAD could increase the content of lauric acid in the digesta. This would be of great interest because lauric acid is one of the most efficient organic acids for inhibiting culture growth of *Campylobacter jejuni* in vitro [2]. *Campylobacter jejuni* is the most important cause of bacterial food-borne enteritis in human beings in Germany [3]. Most infections are linked with consumption and handling of poultry meat. To control *Campylobacter* spp. infections at the level of poultry production feed additives are of interest. The aim of this study was to investigate whether PKFAD as a feed ingredient increases the content of lauric acid in the digesta as a basis for future in vivo studies on possible antimicrobial effects of lauric acid on Campylobacter.

Methods: A total of 34 CobbSasso 175 broiler chicken (CS) were randomly divided in two groups. Each group (17 birds) of 2-week-old chickens were reared in boxes littered with wood shavings for 28 days. Both groups were fed ad libitum. One group got a diet containing a conventional plant fat mixture (12.7 MJ ME/kg diet; 189 g XP/kg diet; 81.0 g XL/kg diet; 0.82 g Na/kg diet; 6.46 g K/kg diet, 0.40 g lauric acid/kg diet). The other group was fed the same conventional diet in which 5.00 % of the plant fat mixture was substituted by PKFAD (12.6 MJ ME/kg diet; 193 g XP/kg diet; 77.0 g XL/kg diet; 0.82 g Na/kg diet; 7.00 g K/kg diet, 18.8 g lauric acid/kg diet). Five birds of each group were slaughtered at d 43. The gastrointestinal tract was carefully removed to collect the contents of the crop, stomach, proximal small intestine (stomach to Diverticulum vitellinum), distal small intestine (Diverticulum vitellinum to caeca) and caeca. The lauric acid content was measured in the sampled freeze dried digesta by gas chromatography. The differences between the groups concerning lauric acid content in the digesta were tested using the t-test for the normal distributed and the Wilcoxon-test for the not normal distributed data (significance level: $p < 0.05$).

Results: In comparison to the conventional fed group the PKFAD fed group had a significant higher content of lauric acid in the digesta of all measured intestine sections. The content of lauric acid in the digesta decreased from crop to distal small intestine/caeca in both groups.

Table 2: Averages (± standard deviation) of DM and lauric acid content in the gastrointestinal tract

	DM (g/kg digesta)		lauric acid content (g/kg digesta)	
diet	plant fat mixture	5 % PKFAD	plant fat mixture	5 % PKFAD
crop	346 ±13.9	296 ±76.0	0.22 ±0.01^{b}	6.41 ±2.34^{a}
stomach	366 ±45.0	355 ±13.1	0.19 ±0.05^{b}	5.53 ±1.91^{a}
proximal small intestine	153 ±20.6	147 ±21.9	0.07 ±0.04^{b}	1.68 ±0.26^{a}
distal small intestine	172 ±18.5	177 ±21.0	0.03 ±0.01^{b}	0.88 ±0.39^{a}
caeca	172 ±21.8	203 ±9.86	0.06 ±0.04^{b}	0.11 ±0.04^{a}

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

Conclusion: These results confirm the hypothesis of the study that PKFAD as a feed ingredient significantly increases the content of lauric acid in different parts of the gastrointestinal tract. Because of the proven higher content of lauric acid in the digesta of animals of the PKFAD fed group, further studies to investigate possible influences of a PKFAD enriched diet on an experimental *Campylobacter jejuni*-infection would be of great interest.

[1] (NANDI et al. 2005): Enzyme and microbial technology 36, 725-728
[2] MOLATOVÁ, Z. (2010): Folia microbiologica 55, 215-220
[3] RKI (2015): Epidemiologisches Bulletin 39

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139.

Effect of conjugated linoleic acids on metabolism of tocopherol in lactating rats and tissue tocopherol concentrations in their offspring

Effekte konjugierter Linolsäuren auf den Stoffwechsel von Tocopherol bei laktierenden Ratten und die Tocopherolkonzentrationen in Geweben der Nachkommen

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Conjugated linoleic acid (CLA) is known to affect the lipid metabolism in growing and lactating animals and humans (1). However, potential effects on the metabolism of fat-soluble vitamins in lactating animals and co-occurring effects on their offspring are unknown. Therefore, the aim of this study was to investigate effects of dietary CLA on concentrations of tocopherol in tissues of lactating rats and their offspring and to determine expression of genes involved in its metabolism and transport.

Methods: Twenty-eight female Wistar Han rats were allocated to 2 groups and fed a control diet (control group) or the same diet with 1.5% of sunflower oil replaced by a CLA preparation (Lutalin, BASF) supplying each 0.45% of cis-9, trans-11 and trans-10, cis-12 CLA to the diet (CLA group) during pregnancy and the first 14 days of lactation. Feed intake of dams and body weight of dams and their pups were recorded weekly. In accordance with the German Animal Welfare Law, the animals were killed for scientific purposes (JLU No. 480_M) and samples of lung (pups only), plasma, gastrocnemius muscle, mammary gland (dams only) and of liver and adipose tissue were taken, frozen in liquid N_2 and stored at -80°C. Plasma and tissue tocopherol concentrations were determined at day 14 of lactation in dams and 1, 7 and 14 days after birth in pups (2). Gene expression of CYP3A1, a cytochrome P450 enzyme involved in tocopherol metabolism and of α-tocopherol transfer protein (TTPA) was determined in dam liver, and low density lipoprotein receptor (LDLR), scavenger receptor class B member 1 (SCARB1), and lipoprotein lipase (LPL), involved in tocopherol transport, in dam liver, mammary gland and adipose tissue (2). The data were statistically analysed by analysis of variance considering treatment and, if applicable, time and their interaction as fixed effects.

Results: Feed and tocopherol intake and body weights of pregnant and lactating rats were similar in both groups. Body weights of pups nursed by control and CLA-fed dams were similar at birth (7.1±0.7 and 7.2±0.7 g, respectively) (mean±SD), 7 days (17.9±1.8 and 18.6±1.7 g) and 14 days after birth (38.2±3.7 and 39.2±3.1 g) ($P>0.10$). Tocopherol concentrations in dam plasma (Control: 22.1±4.5 and CLA: 24.5±2.6 µmol/l) and muscle (45.0±9.3 and 45.3±8.2 nmol/g) were similar ($P>0.10$). However, liver tocopherol concentrations were lower in CLA-fed dams (374±58 nmol/g) compared to control dams (491±79 nmol/g) ($P<0.001$) while relative mRNA concentrations of TTPA and CYP3A1 in liver were similar between groups ($P>0.10$). Tocopherol concentrations were higher in adipose tissue of CLA-fed (199±43 nmol/g) compared to control dams (136±25 nmol/g) ($P=0.001$). In addition, relative mRNA concentrations of receptors involved in tocopherol uptake were increased by 30% (SCARB; $P=0.01$) and 50% (LDLR, $P=0.04$) in the adipose tissue in response to CLA-feeding, but expression of LPL was not influenced ($P=0.54$). In the mammary gland, relative mRNA concentrations of LPL, LDLR and SCARB1 were similar in both groups. Additionally, tocopherol concentrations at days 1, 7, and 14 in the milk curd removed from the pup´s stomachs, and in liver and lung, were not influenced by CLA feeding of dams. Adipose tissue tocopherol concentrations were similar at 7 days of age (Control: 52.2±11.2; CLA: 50.9 9.1 nmol/g) ($P > 0.10$), but higher in 14-day old pups nursed by CLA-fed (72±14 nmol/g) compared to control dams (49±6 nmol/g) ($P<0.001$).

Conclusion: We show that dietary CLA affect tissue tocopherol concentrations in lactating rats and alters expression of genes involved in tocopherol transport, but had little effect on tissue tocopherol concentrations in their offspring. This indicates that moderate dosages of dietary CLA in pregnant and lactating animals and humans may be uncritical considering the tocopherol status of new-borns.

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140.

Influence of conjugated linoleic acids (CLA) and vitamin E on the energy metabolism of transitional dairy cows

Einfluss von konjugierten Linolsäuren (CLA) und Vitamin E auf den Energiestoffwechsel peripartaler Milchkühe

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During the transition period dairy cows are confronted with profound changes in their metabolic status due to the onset of lactation and a low feed intake. This often results in a negative energy balance, which may lead to ketosis and oxidative stress. It has been shown that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid (CLA) induces a reduction of *de novo* milk fat synthesis in the udder and thereby might reduce the negative energy balance by reducing the milk energy output. The aim of the experiment was to investigate the anti-ketogenic effect of CLA and the interactions between CLA and vitamin E.

Methods: Fifty nine pluriparous German Holstein cows were allocated to four groups six weeks *ante partum* (a.p.). As especially cows with a higher body condition score (BCS) prior to calving are predisposed for developing a ketosis in early lactation only cows with higher BCS were included into the study. The average BCS was higher than 3.5 at day 42 a.p. in the three treatment as well as in the control group (n=16). The CLA group (n=16) received 8 g *trans*-10, *cis*-12 CLA/d (BASF Lutrell®). The vitamin E group (n=15) received 3,000 IU vitamin E/d (BASF Lutavit® E 50), while the CLA + vitamin E (n=12) group got both supplements. Prior to calving all cows received an energetic oversupply at 60% concentrate in order to further increase the ketotic predisposition. After parturition the concentrate to forage ratio increased stepwise until day 21 *post partum* (p.p.) from 30:70 to 50:50. The BCS was assessed weekly. Blood samples were taken on day 42, 14, 7, 3 a.p. as well as on day 1, 3, 7, 10, 14, 21, 28, 35, 42, 56 and 70 p.p.. The samples were analyzed for non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB). The statistical analysis was performed using the MIXED procedure of the SAS software (9.4) for repeated measures with a compound symmetry structure. The factors in the model were experimental treatment, time and the interaction between them. *P*-values < 0.05 were considered to indicate significant differences.

Results: The milk fat percentage was lower in the CLA group compared to the control group from lactation week 3 to 9 ($P < 0.05$; Fig. 1). However, the milk fat percentage in the CLA + vitamin E group was reduced in lactation week 9 only ($P < 0.05$). Due to counterbalancing effects of milk yield and milk energy output the energy balance was not significantly influenced by treatments. NEFA and BHB concentrations in the blood serum showed no significant differences between groups (Fig. 2).

Conclusion: CLA reduced the milk fat content as expected. It did not mitigate the negative energy balance in cows prone to ketosis. However, since the control group did not get into ketosis, treatment effects could not be demonstrated. Vitamin E, however, seems to counteract the CLA effect on the milk fat content to a remarkable extent and also seems to reverse effect CLA has on the milk yield. No differences in milk energy output were found.

Fig.1 Milk fat content (LSMeans ± SEM)

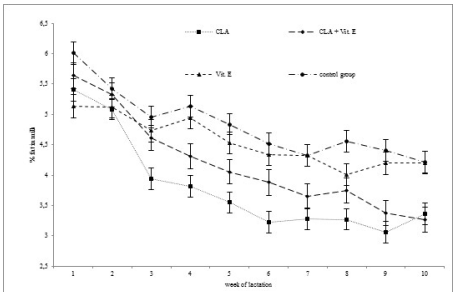
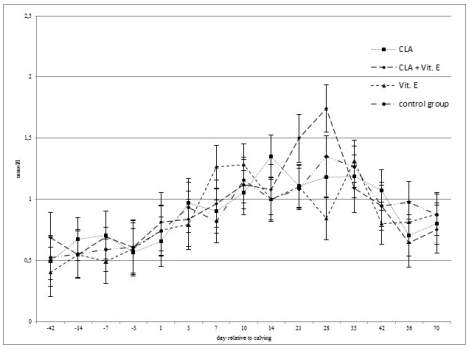


Fig.2 BHB-levels in blood serum (LSMeans ± SEM)



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141.

Maternal supplementation with fish oil improves insulin sensitivity and up-regulates PPARγ gene expression in the liver of post-weaned rabbits

Eine Integration der mütterlichen Ernährung mit Fischöl erhöht die Insulinempfindlichkeit und Genexpression PPARy in der Leber von entwohnten Kaninchen

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Recent research suggests that eicosapentanoic (EPA) and docosahexaenoic acids (DHA), contained in fish oils, improve insulin sensitivity in humans and animals (1). There is also evidence that fish oil supplementation during pregnancy and lactation may influence glucose metabolism of offspring via *developmental programming* (2). Peroxisome proliferator-activated receptor gamma (PPARγ) is a lipid sensitive receptor that regulates many physiological processes including glucose, lipid metabolism, and reproduction (3). PPARg may be involved in the beneficial effects induced by EPA and DHA (1). The objective of this work was to evaluate the long-term, transgenerational effects of maternal supplementation with fish oil on insulin sensitivity and liver expression of PPARγ of post-weaned rabbits as a model to study translational metabolic programming.

Methods: Thirty multiparous hybrid rabbit does were randomly subdivided in two groups and fed either a control diet (C group, n=15) or an iso-energetic diet supplemented with 3% fish oil (Nordos ® - FO group, n=15). Nutritional treatment of does begun 2 months before insemination and continued until weaning (35th day post-partum), when young rabbits were separated from their mothers and moved into individual cages. Thereafter, all weaned rabbits were fed the same control diet. From days 35 to 80 of age, food intake was recorded daily and BW weekly. At day 80, blood sampling and intravenous glucose tolerance test were performed on 5 male rabbits/group randomly selected according to the mother's diet. Rabbits were fasted overnight. A bolus of glucose (0.6 g/kg) was infused into the ear marginal vein. A drop of capillary blood was collected by skin puncture of the ear just before glucose administration and then after 5, 10, 30, 60, and 120 min. Blood glucose was measured by a calibrated glucometer and insulin concentrations by RIA. The homeostasis model assessment for insulin resistance (HOMA-IR) and kinetic parameters of glucose tolerance test were calculated. To evaluate the expression of PPARg by RT-PCR, total RNA was extracted from three rabbit livers from each group (C and FO). Primers for PPARg were (Genbank n. AF013266): sense 5′-TGAAGGATGCAAGGGTTTCT-3′ and antisense 5′-CCAACAGCTTCTCCTTCTCG-3′.

Results: There were no differences in BW (2.2±0.2 vs. 2.3±0.2 kg, P = 0.8) or food intake (218±3 vs. 214±3 g/d, P = 0.2) between C and FO groups, respectively. However, maternal supplementation with fish oil reduced fasting glucose concentrations (7.1±0.3 vs. 5.8±0.5 mmol/l on C and FO, respectively; P = 0.015) and improved response to glucose load compared to C offspring. The glucose elimination rate constant was higher (P=0.039) in the FO group (1.0±0.0%/min) than in C group (0.7±0.2%/min). Glucose concentrations, 2 hours after the load, were lower (P=0.047) in the FO group (5.6±0.3 mmol/l) than in C group (6.8±0.6 mmol/l). Insulin concentrations (5.9± 0.6 µU/ml and 6.6± 2.7 µU/ml on C and FO groups, respectively; P = 0.8) and HOMA-IR (1.7±0.2 and 1.5±0.6 on C and FO, respectively; P = 0.9) did not differ between groups. Fish oil supplementation up-regulated (P≤0.01) PPARg mRNA expression in post-weaned rabbit livers.

Conclusions: Maternal fish oil supplementation improved insulin sensitivity, mainly under dynamic conditions, and up-regulated PPARγ gene expression in the liver of post-weaned rabbits. These findings indicate that maternal EPA and DHA improved insulin sensitivity of offspring possibly through PPARγ involvement.

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Workshop: Grain in animal feed – Insights and Perspectives

Workshop: Getreide in der Tierernährung –Erkenntnisse und Perspektiven

WS1 Chemical processing of cereal grains in ruminant nutrition: between tradition and innovation

Chemische Behandlung von Futtergetreide in der Wiederkäuerernährung: Zwischen Tradition und Innovation

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Cereal grains such as maize, barley, wheat, triticale, and sorghum have long become an important component of the diet in high-producing ruminants. Cereals are rich in energy and their inclusion in the diet increases the energy intake, supporting high milk production or rapid growth rates. Starch is by far the main component of cereals and its utilization is responsible for the feeding value of cereals in ruminants. Besides starch, grains also are a good source of proteins and minerals, especially of phosphorus (P) in ruminant diets. To improve degradation and utilization of grain starch, and also optimize the feeding value of cereals in ruminants, grains typically are processed. However, starch is very variable from cereal to cereal in terms of its chemical structure and digestive properties. Therefore, one processing technique cannot be used with the same aim in all types of cereals. Main methods used in grain processing in ruminants are physical and chemical methods, which can be applied singly or in combination. The former includes techniques that break the outer tissues of the grain to allow access of rumen microorganisms and digestive enzymes, e.g. grinding, rolling and crushing, as well as methods that affect the susceptibility to microbial attack in the rumen and enzymatic action in the intestine, e.g. through heating in addition to rolling to cause gelatinization of starch. Mechanical or thermal treatments, such as steam flaking, roasting, popping, reconstituting or micronizing, were mostly performed in order to enhance the starch digestibility in the rumen and to increase the feed utilization efficiency. However, the negative effects of feeding diets rich in degradable starch, such as high incidences rumen disorders, which were observed in the intensive rearing systems of ruminants from the eighties onwards, changed this trend of grain processing in cattle. For this reason, many attempts have been made to develop grain processing technologies to promote the animals' performance and feed utilization, but without impairing animal health.

On the other hand, chemical treatments with alkali, e.g. sodium hydroxide (NaOH) or ammonia, brings about an effect similar to that of rolling or crushing in enabling access of rumen microbes and digestive enzymes. However, although processing is essential to maximize the utilization of grains by ruminants, extensive grain processing increases ruminal starch degradation, which may negatively affect feed intake in ruminants as this process also enhances the risk of rumen fermentation disorders. Thus, the development of effective feeding strategies for ruminants requires the maintenance of an optimal rumen metabolism. As slowing down the rate of ruminal degradation of starch-rich cereals would reduce the starch availability for microbial degradation in the rumen and shift some starch digestion to the small intestine, this would minimize the risk for rumen fermentation disorders.

In this regard, treating of whole grain with chemicals to increase digestibility of the seed coat and subsequently whole grain digestion within the rumen has been discussed as one strategy to reduce processing costs (for grinding) as well as to slower the rate of starch digestion in the rumen to improve fibre digestion with potential beneficial effects on intake and animal production. Furthermore, treatment of milled grains with chemical agents has been applied to retard the rate of digestion in the rumen of cereal starch and protein. Thus, effects of some traditional as well as more innovative chemical processing techniques are discussed in the following.

Among the "traditional" chemical grain processing methods considerable research has been conducted on the NaOH treatment of grain, and there is sufficient evidence from cattle experiments to indicate that digestibility, weight gain, and milk production on NaOH-treated whole grain can be similar to that on rolled

grain, provided sufficient NaOH is applied. Treating milled grains with NaOH has been shown to result in a slower ruminal starch and protein degradation as well as decreased susceptibility to rumen acidosis. On the other hand, total tract digestibility has been shown to be enhanced (barley) or even reduced (sorghum), thus indicating that the type of grain itself also has to be taken into consideration when processing grain with NaOH. However, a number of practical and commercial considerations have limited the more widespread adoption of this technology on farms. For instance, negative side effects (e.g. nephrotoxicosis after prolonged feeding of high NaOH-amounts, soil salinification, risks for users as well as adverse effects on nutritional quality) preclude its application as routine technique in the practice.

Another chemical that has been widely used to treat grains is formaldehyde (HCHO), which retards the rate of digestion in the rumen of cereal starch and protein, without hampering total tract digestion and may have beneficial effects on forage intake. Nevertheless, also environmental and health issues have to be considered thoroughly when using HCHO as a chemical to treat feedstuffs for animal production.

Besides NaOH and HCHO, treatment of grain with ammonia has been used to render ruminal starch degradation of grains, i.e., to decrease the rate of starch and protein degradation in the rumen. Although ammonia treatment offers a more practical alternative to NaOH and HCHO, digestibility and animal production responses have been highly variable, and research is required to identify effective ammoniation procedures.

Besides, the potential of the aforementioned chemical agents to render ruminal starch and protein degradation of grains in a positive manner, some of this treatments, e.g. HCHO, have been shown to coincidentally reduce the phytate solubility and thus the availability of P in the rumen, that might impair the total tract digestibility of P. Thus, the positive effect of enhanced nutrient flow to the intestines is counteracted by the decreased utilization of phytate-bound P. Nevertheless, contradictory effects have been observed, as the results of chemical processing of grain depend on the type of chemicals used, their concentration, as well as the type of the grain treated itself.

Despite the advantages provided, the use of NaOH, HCHO and ammonia treatments require long soaking periods, thus being very laborious and intensive methods. Furthermore, along with health consequences when applied during long periods of time, some of these traditional methods are corrosive, pose health risks to laborers and possibly even consumers.

In recent years there has been an increasing interest to identify new chemical grain processing techniques. Such “innovative” chemical grain processing methods include the treatment of grains with mild acids, in order to modify the chemical characteristics of the grain.

Previously, treatments with organic acids (especially propionic acid) have been mainly used to enable storage of high-moisture grain to save drying costs or allow grain to be harvested at some earlier time, respectively. Furthermore, especially organic acids, that are found in biological tissues or produced in the gastrointestinal tract, are generally used to modify rumen fermentation. Among them, fumaric, malic and aspartic acids were, so far, the most frequently used acids in ruminant nutrition, with malate showing the most promising effects, such as increasing the dry matter digestibility, decreasing methanogenesis and uncomplicated action. Nevertheless, besides the high cost, none of the above mentioned acids have been evaluated in terms of slowing down ruminal starch degradation. Also, in situ trials with tannic acid have shown its potential to decrease ruminal dry matter and protein degradation of barley grain in the rumen. Nevertheless, intensive in vivo research is lacking to evaluate the potential of this technology for practical application.

Recent studies investigated the effects of treating grains with other organic acids such as lactic or citric acid, two acids that are widely used as a low-cost means of food preservation by different industries. In vitro studies have demonstrated modulation of the chemical composition of barley treated with these acids. Feeding dairy cows barley grain steeped in lactic acid has been shown to exert some beneficial effects, such as decreasing the starch degradation rate in the rumen, modifying ruminal fermentation and enhancing the energy status of the animals. In contrast to the classical chemical processing techniques, that might even impede P utilization in ruminants, it has been observed, that lactic and citric acid treatments of cereals trigger

the hydrolysis of native phytate and enhance ruminal P-disappearance. However, further investigations are required to elucidate the mechanisms behind the phytate degradation and further in vivo trials are required to tap the potential of this technique for reducing inorganic P supplementation and P excretion.

In conclusion, processing techniques that enhance the nutritive value as well as ruminal tolerance of grains are becoming increasingly important, not only in terms of lowering the risk of metabolic disorders and promoting digestion, but also in enhancing the nutrient supply for the host ruminants. Despite the progress made in using various processing methods, the impact of grain characteristics, both physical and chemical, on the response to chemical treatments requires further research. Furthermore, it is important to optimize concentrations of chemicals and also reveal their mode of action in the host digestive tract.

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Fermentation of grain: potentials in pig nutrition

Fermentation von Getreide: Potentiale beim Schwein

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The term fermentation (lat. fermentum "fermentation") can be referred to as the metabolism of organic substrates by bacteria, fungi, or cell cultures. The most well-known form of fermentation – besides the production of ethanol – is the preservation of feeding stuffs by ensiling. In addition, with the help of this biotechnological process a broad range of products is produced such as enzymes, antibiotics, amino acids or bio ethanol. In agriculture, the fermentation process is used for preservation purposes, reduction of harmful microbes, or also for improving nutrient digestibility in animal feed. The impact of fermentation on the content of antinutritional compounds, such as phytates, trypsin inhibitors, allergens, saponins or tannins, has also been reported (1). Moreover, a high-quality fermentation process can reduce the pH in feed as well as the gastrointestinal tract, and it may activate naturally occurring plant enzymes and improve gut health (2).

Basically a distinction between the fermentation of liquid feed and the fermentation process during wet storage conditions of grains (ensiling), should be done for animal nutrition. Regarding the fermentation of liquid feed mixes a "directional" fermentation, which takes place by the addition of specific lactic acid bacteria at a controlled temperature/humidity environment for a certain period of time, should be preferred over an "uncontrolled" feed fermentation process (3). In addition, experiments showed that a fractional fermentation of feed materials or the grain group alone, positively impacted the zootechnical parameters of the animals, affected by a better nutrient digestibility and a reduction of antinutritional compounds, compared to the fermentation of a total feed mix (4). A plausible explanation for this phenomenon seems to be the decarboxylation of free amino acids contained in total feed rations. The reduction can amount up to 40% of the occurring free amino acids in the total feed mix. As a consequence, an adequate supply with lysine to meet the animal's requirement cannot be ensured (5). Furthermore, a decrease in daily feed intake affected by the increased levels of biogenic amines, such as cadaverin and the associated impaired taste, can be a consequence (5).

Besides the fermentation of liquid feed, storage of grains in a wet form to save drying costs and hence feeding of fermented grains (grinded or as whole grain) is widely spread in swine production in some regions of Europe. Maize is one of the most important feed ingredients in diets for pigs. Scientific studies report on a potential to improve the nutrient digestibility of pig diets by using fermented maize as the main feed component (6, 7). Based on these studies, it can be assumed, that the digestibility improving effects are more pronounced in the grinded maize silage compared to maize stored as whole grain. Furthermore, fermented maize may require additional adjustments to its amino acid supply (6, 7). Similar results as for maize were observed for the industrial by product wheat bran, applying solid state fermentation (8). Hence, fermentation offers some potential to increase the proportion of wheat bran in pig diets by improving the digestibility of organic/inorganic nutrients and energy. From a sustainability perspective, using high amounts of industrial by-products would be desirable, in order to reduce the strong competition for food between humans and monogastric livestock.

Processing techniques and storage conditions including fermentation processes may exert effects on phytate as well as other inositol phosphates. The acidification of the diet/feed stuff, affected by the fermentation process can lead to activation of endogenous plant phytase (9). Furthermore, lactic acid bacteria are able to promote phytate degradation due to their ability to produce phytase (9). In recent years, fermentation of dry feeds was effectively used to degrade phytate P and improve mineral and nutrient digestibility in diets for pigs. A fact that needs to be considered is the dry matter content of the diet/feed stuff during the fermentation process. In general, a higher moisture content in feed increases fermentation capacity and phytate hydrolysis (6, 7, 9).

However, as fermentation progresses are dynamic, varying microbial and nutritional characteristics result, leading to different effects on nutrient digestibility. Hence, further research is needed for the optimization of the different fermentation techniques in respect of swine nutrition.

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Cereal grains as source of protein and amino acids in livestock feeding

Getreide als Protein- und Aminosäurenquelle in der Fütterung

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Cereal grains are usually considered as energy source in livestock feeding. Concentrations of crude protein (CP) and amino acids (AA) are in general relatively low; however, at high inclusion rates of cereal grains in the diet or ration – as often used – grains contribute significantly to the CP and AA supply of livestock. This was a motivation for the GrainUp consortium to systematically characterize the protein value of cereal grains (www.grain-up.de). This contribution gives an overview of the main findings.

Different grain species including barley, maize, rye, triticale and wheat were used, grown in the same year and location and differed in their genotype only [1]. Concentrations of CP and AA varied between species but were in general within the range of values published in feed tables (Table 1). Ruminal degradation characteristics were studied in 100 grain samples (5 species with 20 genotypes each) using the in situ technique and rumen fistulated Jersey cows. The standardised precaecal AA digestibility was determined in 32 grain samples (4 species with 8 genotypes each) using cannulated pigs, and based on the protocol as recommended by GfE [2]. Amino acid digestibility was also determined in caecectomised laying hens [3] using 80 grain samples (4 species with 20 genotypes each). Results of all studies are summarised in Table 1.

Crude protein degradation in the rumen

The potential rumen degradation of CP was almost complete (> 95%) in all assay grains. However, differences existed between the estimated parameters of degradation (a, b, c), both between and within one grain species. The CP degradation was very slow in maize and very fast in rye, with values for triticale, wheat and barley being intermediate and similar. The EDCP5 estimate was on average 85 % for the soft grains. The estimates of the effective degradability of CP calculated for a rumen outflow rate of 8 %/hour (EDCP8) were 3 to 9 % lower than EDCP5 estimates, and differences were greater for barley and wheat than for rye and triticale. The EDCP estimates of maize were substantially lower than EDCP estimates of the soft grains. In general, inter-genotype variation in EDCP estimates of soft grains was low due to fast degradation. In contrast and as a consequence of the slower degradation, EDCP estimates for maize differed to a greater extent between genotypes. There was a strong negative correlation between EDCP values of maize genotypes and their CP concentration ($P < 0.001$) [4]. Negative correlations between EDCP and the concentration of specific AAs (Pro, Glu, Ala, Leu and Phe) associated with storage proteins (prolamins and glutelins) were also significant ($P < 0.05$), while typical AAs of metabolic proteins (albumins and globulins) (Asp, Gly, Lys, Arg, Trp) were positively correlated with the EDCP values ($P < 0.001$). These results indicated that differences in EDCP of maize were caused by differences in the proportions of protein fractions between genotypes.

In conclusion, differences between maize genotypes should be considered in ration formulation. However, when using soft grains the use of mean values of EDCP as reported in Table 1 is acceptable for practical application. It was not possible to study degradation characteristics of oat grain by using the in situ technique due to very high washout losses [5].

Amino acid digestibility in pigs and hens

In pigs, the digestibility of CP and AAs of triticale and wheat was overall higher than values observed for barley and rye. When compared with values in current feed tables, digestibility values were lower in all grain species assayed in the GrainUp project. However, it should be noted that digestibility values from this study are similar to or even greater than those recently published from other projects for different grain species [e.g. 6, 7]. This calls for a revision of feed tables with regard to precaecal AA digestibility values of cereal grains.

In laying hens, AA digestibility overall was high, with the exception of rye. Amino acid digestibility of rye was much lower than for the other grains and also much lower when compared to the digestibility values determined in pigs. Digestibility values of triticale were closer to values observed for wheat than for rye.

The project also revealed significant differences in digestibility values of CP and AA between genotypes of one grain species in both pigs and hens. However, this inter-genotype variation was much greater in hens than in pigs. This suggests that knowledge about batch-specific AA digestibility values is more important in feed compounding for hens than for pigs.

Table 1: Overview of analytical, degradation and digestibility data from the GrainUp project (mean and SD)

		Barley		Maize		Rye		Triticale		Wheat	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Analysed concentrations											
No. of genotypes:		21		27		22		21		29	
CP	g/kg DM	123	7	94	9	117	5	124	7	137	9
Lys	g/16 g N	3.5	0.17	3.0	0.23	3.6	0.10	3.2	0.15	2.7	0.12
Met	g/16 g N	1.6	0.06	2.1	0.23	1.5	0.04	1.6	0.05	1.5	0.05
Thr	g/16 g N	3.4	0.12	3.7	0.07	3.2	0.06	3.1	0.09	2.9	0.07
Trp	g/16 g N	1.2	0.05	0.8	0.06	1.0	0.03	1.1	0.07	1.2	0.05
Ruminal CP degradation characteristics[1]											
No. of genotypes:		20		20		20		20		20	
a	%	26	3	23	4	32	2	31	4	17	4
b	%	72	3	77	4	63	2	66	4	81	4
c	%/h	20	3	5.1	0.7	43	4	27	3	21	2
$EDCP_5$	%	83	2	62	4	88	0.5	86	0.9	82	2
$EDCP_8$	%	77	3	53	4	85	0.7	82	1.3	76	2
Standardised precaecal digestibility in pigs											
No. of genotypes:		8 [8]		not studied		8 [9]		8 [10]		8 [11]	
CP	%	72	2			73	1	83	1	84	1
Lys	%	64	2			62	2	74	2	71	2
Met	%	77	2			75	1	85	1	86	1
Thr	%	71	1			64	1	75	1	79	2
Trp	%	70	2			65	1	81	1	82	2
Digestibility in caecectomised laying hens											
No. of genotypes:		not studied		20 [12]		20 [13]		20 [14]		20 [15]	
Lys	%			79	4	49	6	74	3	80	6
Met	%			91	2	67	5	83	3	84	8
Thr	%			83	4	45	6	73	3	82	5
Trp	%			69	14	56	8	79	3	84	4

1 a, b, c were calculated from the equation Deg = a + b × (1 - e-c×t), where Deg (%) = degradation after t hours; a = washout fraction; b = potentially degradable fraction; c = rate of degradation of b and t = time (h). Effective degradability of crude protein was calculated using the equation EDCPk = a + [(b × c) / (c + k)], with k = ruminal outflow rate (5 and 8 %/h).

Conclusions

Cereal grains are mainly considered as a source of energy in livestock feeding. However, they also contribute extensively to the animal's protein supply. The GrainUp project systematically characterised the ruminal degradation of CP, and the precaecal digestibility of AA in pigs and laying hens. The consideration of grain species-specific degradation and digestibility values in diet formulation will contribute to an improved efficiency of protein utilisation. Intra-species differences are especially relevant in maize grain (protein degradation in the rumen) and for all assayed grains fed to laying hens.

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Upgrading of cereals by means of hydrothermal pressure treatments

Veredelung von Getreide durch druckhydrothermische Verfahren

*Graf von Reichenbach H. – Düsseldorf

1. Einleitung

Komplette Futtermischungen oder Einzelkomponenten wie Getreide lassen sich druckhydrothermisch durch zeitliche Einwirkung von Feuchtigkeit, Temperatur, Scher- und Knetkraft, Druckaufbau und Druckentlastung zu Halbfabrikaten oder Produkten mit verbessertem Nährwert und ggf. funktionalen Eigenschaften veredeln. Das Autoklavieren, Flockieren, Expandieren oder Extrudieren hat Auswirkungen auf alle Inhalts-stoffe. Stärke wird aufgeschlossen, die Faltungsstruktur von Proteinen wird modifiziert; manche antrinutritiven Faktoren werden eliminiert, und auch die Rohfaser, darunter die Strukturkohlen-hydrate, wird in ihrer Struktur und Verdaulichkeit beeinflusst. Die Quellfähigkeit, Löslichkeit, Verdaulichkeit und Verdauungskinetik der Inhaltsstoffe ändern sich, die Viskosität und Puffer-kapazität des Chymus im Verdauungstrakt kann sich erhöhen. Eine Schädigung von Inhaltsstoffen soll vermieden und deren Effizienz und Nutzungsgrad im Tier verbessert werden.

Immer mehr Aufmerksamkeit gilt der Vermeidung nachteiliger Folgen bzw. der Verbesserung tiergesundheitlicher Aspekte durch die Futtermittel-Behandlung, welche allerdings zusätzlichen verfahrens- und kostenseitigen Aufwand erfordern kann.

2. (Druck-) Hydrothermische Verfahren:

Der druckhydrothermischen Behandlung von Getreide geht eine Reinigung (Sichtung, Absiebung), evtl. sogar ein Putzvorgang voraus. Auch kann eine Fraktionierung bzw. Aufkonzentrierung von Rohfaser, Stärke und Protein durch Schälung der Saat, Entkeimung und/ oder eine Separierung der Aleuronschicht vom Getreide-Endosperm vorgenommen werden. Es folgt eine Vermahlung der Rohware mit Hammermühle oder Brechwalzenstuhl, um ein bestimmtes Mahlspektrum, d.h. eine gewünschte Partikelgrößenverteilung einzustellen. Zum Flockieren wird Getreidesaat grob und gleichförmig gebrochen, zum Expandieren oder Extrudieren meist fein ver-mahlen. Daran schließt sich die eigentliche druckhydrothermische Behandlung an, die auch mehrere Stufen umfassen kann – z.B. Vorkonditionierung, Expansion und Pelletierung.

Die Vorkonditionierung erfolgt für gewöhnlich kontinuierlich in einem Durchlaufmischer mittels Sattdampf. Sie dient als Vorbehandlung (Vorkonditionierung) für die anschließende druckhydro-thermische Behandlung. Das zerkleinerte Getreide wird vorerhitzt und durch den in der Rohware kondensierenden Dampf gleichmäßig befeuchtet. Eine Absteh-Phase in einem Durchlauf-Verweil-behälter kann sich anschließen.

2.1 Konditionieren / Dämpfen

Ein Dämpfprozess unter atmosphärischem Druck oder bei Überdruck kann bereits zur Herstellung von Endprodukten (z.B. von Parboiling-Reis oder „getoastetem" Vollfettsoja) dienen. Dämpfen und Autoklavieren sind vor allem in der Lebensmittelindustrie bekannt und können absätzig (chargen-weise) oder kontinuierlich erfolgen. Gedämpftes Getreide läßt sich heiß zu Grütze oder Kuskus schneiden. - Ein spezielles Verfahren stellt das Puffen dar: Unter hohen Dampfdruck gesetztes Getreide wird schlagartig freigesetzt und schäumt durch die rapide Wasserverdampfung im Korn-inneren zu stark aufgeschlossenen Produkten, z.B. zu Puffreis oder Popcorn auf.

2.2 Flockieren

Das Flockieren setzt eine gezielte grobe Zerkleinerung des zuvor genetzten Getreides und eine heiße Vorkonditionierung mit nachfolgender Abstehdauer voraus, die zu einer Quellung und zu partiellem Aufschluss der Stärke führt. Die feuchtheißen Partikel werden dann im Flockierstuhl zwischen zwei Glattwalzen zu dünnen Flocken gequetscht. Hierdurch wird die Morphologie der gequollenen Stärke-Granula gestört, so dass diese beim anschließenden Abkühlen und Trocknen nicht wieder in ihre ursprüngliche Form und Struktur zurückfinden können. Je dünner die Flocken, desto größer der Stärkeaufschlussgrad (**, der für gewöhnlich 50% unterschreitet. Ein Trocknungsprozeß schließt sich an.

2.3 Pelletierung
Statt des Flockierens kann auch eine Pelletierung den Stärkeaufschluss intensivieren und stabilisieren. Neben dem Durchpressen des zerkleinerten vorkonditionierten Getreides durch die Matrizen-bohrungen bewirkt die quetschend-mahlende Wirkung der Kollerrollen auch die gewünschte Störung der Stärkegranula-Struktur sowie eine mechanische Einwirkung auf die Faserfraktion (Spelzen, Schalen, Kleie).

2.4 Autoklavieren / Überdruck-Dämpfen
Diese Druck-Kochbehandlung entspricht einem Koch- oder Dämpfungsprozeß in einem Papin'schen Dampfdruck-Kochtopf. Sie intensiviert den Stärkeaufschluss und verkürzt die Behandlungsdauer im Vergleich zum Garen bei Umgebungsdruck. Die reine Druckkochbehandlung kann absätzig oder auch kontinuierlich erfolgen. Sie ist im Futterbereich bislang kaum etabliert, aber birgt Potential.

2.5 Expansion
Ein Expander besteht aus einem Rohrgehäuse, in welchem eine stabile Welle mit aufgesetzten Schneckenpaddelsegmenten rotiert. Stopschrauben zwischen den Paddelsegmenten oder eine profilierte Rohrwandung verhindern ein Mitdrehen der kontinuierlich zudosierten Rohware und steigern die Knet- und Scherwirkung im Produkt. Hohe mechanische Antriebsenergie wird durch innere Reibung in Wärme umgewandelt, was zu einer zusätzlichen Produkterhitzung auf Tempe-raturen von oftmals über 100 °C führt. – So erfolgt eine kontinuierliche Kurzzeit-Hochtemperatur-behandlung unter Einwirkung von mechanischem Druck, von Dampfdruck, Knet- und Scherkräften sowie schlagartiger Druckentlastung. Nur sekundenlang ist die Rohware hierbei Spitzentemperaturen ausgesetzt.
Der Austritt des Produktes aus der im Querschnitt verstellbaren Expander-Auslaßöffnung hat einen plötzlichen Druckabfall und somit eine schlagartige Wasserverdampfung („flashing") zur Folge, mit erheblichen Einwirkungen auf die innere Produktstruktur. Abhängig von den Prozessparametern werden Stärkeaufschlussgrade(* von über 70% erreicht. Bei üblicher Verarbeitungsfeuchte reicht ein Kühlvorgang mit Umgebungsluft zur Rücktrocknung des Produktes meist aus; bei zu hoher Rest-feuchte muss ein Trockner zum Einsatz kommen.

2.6 Extrusion
Der Aufbau und die Wirkungsweise des Expander und Einwellen-Extruders ähneln einander. Während mit ersterem ein unregelmäßig geformtes schülpiges Produkt (Expandat) erzeugt wird, lassen sich mit dem Extruder definiert geformte Extrudate herstellen; dies bei meist höherer Prozessfeuchte und stärkerem mechanischem Energieeintrag. Mit Zwei- oder Mehrwellen-Extrudern mit ineinander kämmenden Paddelwerkzeugen lassen sich längere Behandlungszeiten, deutlich höhere Temperaturen und höhere Aufschlussgrade als beim Expandieren erzielen. Bei manchen Produkten ist aufgrund hoher Prozessfeuchte der Kühlvorgang mit Umgebungsluft zum hinreichenden Trocknen nicht ausreichend.

3. Behandlungsziele und Behandlungsfolgen:
Stärkeaufschluss gilt als vorrangiger Effekt der druckhydrothermischen Behandlung. Nur sehr bedingt geht damit eine höhere Verdaulichkeit der Stärke einher. Absetzferkel mit ihrer noch unzureichenden Enzymbildung zur Verdauung nativer Stärke profitieren davon. Mais, welchen Wiederkäuer nicht vollständig verdauen, wird durch Aufschluß hochverdaulich. Allerdings verändert die Behandlung die Verdauungskinetik, indem aufgeschlossene Stärke schneller und umfassender im Pansen abgebaut werden kann. Bekannt ist die pansenstabilisierende Wirkung druckhydrothermischer Verfahren auf Proteine. Überbehandlung jedoch kann die Proteinlöslichkeit zu stark herabsetzen, und bei An-wesenheit von reduzierenden Zuckern können Aminosäure-Verluste durch Maillard-Reaktionen eintreten. Vieldiskutiert sind die Behandlungseffekte hinsichtlich der Erhöhung der Chymusviskosität. Versuche mit Masthähnchen zeigten als Ursache eine gesteigerte Löslichkeit der NSP-Fraktion in expandiertem Futter (Graham und Petterson 1992; Son und Ravindran 2012).
In Untersuchungen der Firma DTC (Kleine Klausing 2009) an Suspensionen aus druckhydro-thermisch behandelten Getreidemischungen wurde der Stärkeaufschluss als alleinige Ursache von Viskositätssteigerungen festgestellt. Fledderus et al. (2007) ermittelten bei Ferkeln als Folge einer gesteigerten Chymusviskosität eine langsamere Magenpassage, eine erhöhte Proteinhydrolyse sowie eine signifikant erhöhte Aktivität der Aminopeptidase und Proteinverdaulichkeit. - Die Verwendung druckhydrothermisch aufgeschlossenen

Getreides bietet Möglichkeiten zur gezielten Viskositäts-erhöhung des Chymus. Bei Flüssigfutter mindert aufgeschlossenes Getreide die Sedimentations-neigung von Grobpartikeln und somit die Entmischung. Überdies bilden Spelzen und Schalen des Getreides nach druckhydrothermischer Behandlung keine Schwimmschichten auf Gülleoberflächen.
Druckhydrothermische Behandlung, insbesondere das Flockieren und Pelletieren, bewirkt üblicher-weise eine Nachzerkleinerung der Futtermittelpartikel, was bekanntermaßen nachteilig für die Tiergesundheit und Leistung sein kann (Betscher , 2010; Svihus et al., 2004). Sind jedoch die groben Teilchen in eine Matrix feinerer Komponenten eingebettet, bleibt ihr grobes Vermahlungs-spektrum beim Expandieren weitgehend erhalten. Diesen Umstand kann man z.B. bei der Schweine-futter-Herstellung nutzen: Eine Rezeptur, im wesentlichen z.B. aus feinzerkleinertem Sojaschrot, Sonnenblumenschrot, Vollfettsoja einerseits und sehr grob gebrochenem Getreide andererseits bestehend, lässt sich nach kurzer Vorkonditionierung unter weitgehendem Erhalt der groben Getreide-partikel expandieren. Das Expandat ist hervorragend hygienisiert, es weist für das Schwein eine gesteigerte Rohfaser- und Proteinverdaulichkeit und einen verminderten „Käfig-Effekt“ auf, und die erhaltenen Grobpartikel entfalten ihre positive Wirkung im Gastrointestinaltrakt, wie sie u.a. von Kamphues (2015) für Schwein und auch Masthähnchen beschrieben werden.

4. Ausblick:

Das Potential druckhydrothermischer Behandlungen von Getreide ist noch nicht ausgeschöpft. Durch vorgelagerte Behandlungsschritte, z.B. durch Schälen oder Fraktionieren/Verschieben können Faser, Stärke und Proteine ab- bzw. angereichert und diese Fraktionen nach druckhydrothermischer Weiter-verarbeitung gezielter und tierartspezifischer verwendet werden.
Der Verdaulichkeitssteigerung von Mais für Wiederkäuer durch druckhydrothermische Behandlung kommt mehr und mehr Aufmerksamkeit zu. Hier sollte nach Möglichkeiten gesucht werden, die auf-geschlossene Maisstärke zugleich pansenbeständiger zu machen, wofür unterschiedliche verfahrens-seitige Ansätze bestehen. Druckhydrothermische Verfahren in Verbindung mit der Verbesserung und dem Erhalt von groben Vermahlungsspektren und dem Einsatz höherer Gehalte an Struktur-kohlenhydraten sind noch weiter entwicklungsfähig.

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