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Spongiforme Enzephalopathien bei Mensch und Tier

Groschup, M. H.* – Greifswald – Insel Riems

Scrapie und BSE sind stets tödlich verlaufende neurodegenerative Erkrankungen bei kleinen Wiederkäuern und bei Rindern. Scrapie kann als der Prototyp der transmissiblen spongiformen Enzephalopathien (TSE) bei Mensch und Tier angesehen werden. Diese Gruppe umfasst ferner die Creutzfeldt-Jakob-Erkrankung (CJD), das Gerstmann-Sträussler-Scheinker-Syndrom (GSS), die Kuru und die Tödliche Familiäre Insomnie (FFI) beim Menschen sowie die Chronic Wasting Disease (CWD) bei Zerviden und die Transmissible Nerzenzephalopathie (TME). In Folge der BSE-Epidemie im Vereinigten Königreich kam es zu BSE-Infektionen auch beim Menschen, die sich als Variante Form der CJD (vCJD) manifestierten. Dagegen gibt es derzeit keine wissenschaftlich gesicherten Hinweise auf eine Verbindung zwischen dem Auftreten von CJD-Erkrankungen beim Menschen und Scrapie-Infektionen bei kleinen Wiederkäuern.

Unter dem Begriff ‚Scrapie' wurde bis vor wenigen Jahren eine weltweit verbreitete ‚klassische' Form verstanden. Klassische Scrapie-Fälle werden innerhalb betroffener Herden horizontal übertragen. Hierbei hat der direkte Kontakt mit der Nachgeburt von infizierten Mutterschafen die größte Bedeutung. Auch kontaminierte Weiden, Ausläufe und Ställe können zu einer Infektion bei Schafen führen. In Folge der seit dem Jahre 2002 in der EU intensivierten TSE-Überwachungsprogramme bei kleinen Wiederkäuern wurden neue, bis dato unbekannte atypische Formen der Scrapie gefunden, die sich in ihren biochemischen Eigenschaften und in der Erregerverteilung im Gehirn von den klassischen Scrapie-Fällen unterscheiden. Solche Fälle wurden (in der zeitlichen Reihenfolge ihrer Entdeckung) in Norwegen, Deutschland, Frankreich, Belgien, Schweden, im Vereinigten Königreich, in Portugal, in Irland, in der Schweiz und mittlerweile auch in vielen anderen Ländern gefunden. Klassische und atypische Scrapie unterscheiden sich auch im Hinblick auf ihre Häufigkeit in betroffenen Herden. Während bei der klassischen Scrapie meist mehrere Tiere im Rahmen der obligatorischen Keulung empfänglicher Tiere gefunden werden, werden bei der atypischen Scrapie meist nur Einzeltiere diagnostiziert.
Bei der immunochemischen Charakterisierung des abnormalen BSE-Prion-Proteins der deutschen BSE-Rinder wurden bei vier über acht Jahre alten Tieren atypische BSE-Fälle gefunden. Die zwei unterschiedlichen Immunoblot-Profile für das pathologische Prion-Protein (→ L-Typ und → H-Typ) unterscheiden sich signifikant vom Profil der aus dem Vereinigten Königreich stammenden 'klassischen' BSE-Fälle. Atypische BSE-Fälle kommen in vielen Ländern der Welt vor. Für die deutschen atypischen BSE-Fälle konnte am FLI erstmals gezeigt werden, dass es sich um übertragbare Krankheiten handelt und dass sich ihre Übertragungscharakteristika auf transgene (bovines PrP-überexprimierende) Mäuse von denen bei der 'klassischen' BSE unterscheiden. Es ist nicht ausgeschlossen, dass atypische BSE-Fälle aus spontanen Umfaltungen des pathologischen Prion-Proteins bei älteren Rindern hervorgegangen sind.

Bei Schafen unterscheidet sich die TSE-Pathogenese wesentlich von der bei der BSE bei Rindern. Bei Schafen können nahezu alle Körpergewebe und z.B. auch das Blut bereits wenige Wochen bis Monate nach der Infektion infektiös sein. Deshalb müssen sich die Bekämpfungsmaßnahmen bei den kleinen und großen Wiederkäuern prinzipiell unterscheiden. Während beim Auftreten eines BSE-Falles bei einem Rind die Tötung und unschädliche Beseitigung der Fütterungs- und Geburtskohorte als ausreichend angesehen werden kann, um eine Weiterverbreitung zu verhindern, bedarf ein effizientes Bekämpfungsprogramm gegen die Ausbreitung der klassischen Scrapie und der BSE bei den kleinen Wiederkäuern drastischere Maßnahmen, wie die Tötung und unschädliche Beseitigung aller empfänglichen Tiere in der betroffenen Herde. Angesichts der atypischen Scrapie-Fälle, die sich unter anderem durch abweichende Übertragungs- und Pathogenese-Charakteristika sowie durch eine fehlende genetische Resistenz (aufgrund unterschiedlicher Prion-Genotypen) auszeichnen, erscheinen jedoch auch abgestufte Bekämpfungsmaßnahmen sinnvoll.

Im Vortrag wird ein Überblick über die aktuelle epidemiologische Situation hinsichtlich BSE bei Rindern gegeben. Bisher wurden in Deutschland über 400 einheimische BSE-Fälle diagnostiziert. Die zur Verfügung stehenden Untersuchungsmethoden erlauben bisher lediglich eine Erkennung infizierter Tiere bis wenige Monate vor dem Auftreten klinischer Symptome. Die Entwicklung sensitiverer Testmethoden oder ein Test am lebenden Tier ist nach wie vor wünschenswert. Das Fehlen eines solchen präklinischen Tests ist zumindest teilweise durch die besondere Pathogenese der BSE-Infektion beim Rind begründet: erst unmittelbar vor dem

Auftreten erster klinischer Symptome kann sowohl das pathologische Prion-Protein als auch Infektiosität im zentralen Nervensystem des Tieres festgestellt werden. Es war lange Zeit nicht bekannt, wie der Erreger bei Rindern nach der oralen Aufnahme vom Magen-Darm-Trakt in das ZNS gelangt und wo er sich in den durchschnittlich fünf Jahren der Inkubationszeit im Tierkörper aufhält. Zur Beantwortung dieser Fragen wurde am FLI eine BSE-Pathogenesestudie mit Rindern durchgeführt. Die Ergebnisse dieser Studie belegen den entscheidenden Beitrag des vegetativen Nervensystems bei dem Aufstieg der BSE-Prionen vom Darm in das ZNS.

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1. Reducing human edible inputs in dairy cattle by substituting cereals and pulses with by-products

Reduzierung des humanernährungstauglichen Anteils in Milchkuhrationen durch die Substitution von Getreide und Hülsenfrüchten mit Nebenprodukten

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Question: To meet animals´ high nutrient requirements, rations for high yielding dairy cows include more and more potentially human edible concentrates, which increases the feed vs. food competition and results in low efficiency in terms of net food production of dairy systems. Thus, strategies to reduce human edible inputs in dairy nutrition are warranted. The aim of this feeding trial was therefore, to substitute a mixture of common concentrates with a mixture of by-products from the food processing industry and evaluate the effects of this substitution on milk performance, feed and nutrient intake, blood parameters and the edible feed conversion ratio (eFCR), defined as human edible output via milk per potentially human edible input via feedstuffs, in organic dairy production.

Methods: Eighteen lactating Holstein cows were randomly allotted to either a control (CON) or an experimental group (BP). For CON, the forage mixture (75% grass silage and 25% alfalfa hay on a dry matter basis) was supplemented according to cows´ milk yields with a mixture of concentrates commercially available in organic dairy production (composition: 27% peas, 20% maize, 20% field beans, 16% oats, 13.5% wheat, 3% molasses, and 0.5% commercial mineral and vitamin mixture), while cows in BP received only a mixture of by-products from the food-processing industry as supplements (composition: 41.5% maize middlings, 30.5% beet pulp, 15.5% rapeseed cake, 9% soya cake, 3% molasses, and 0.5% commercial mineral and vitamin mixture). Potentially edible fractions were estimated based on assumptions of Wilkinson (1) with minor modifications and eFCR were calculated on a gross energy and crude protein basis. After the first experimental period, lasting for 7 weeks and including a 2 weeks adaptation period to the diet, cows switched groups (change-over design).

Results: The CON mixture had higher starch, but lower fiber and ether extract contents compared to BP and these differences were also observed in the intake of these respective nutrients. There were no differences in milk yield and milk solids and blood parameters did not indicate negative effects on cows´ metabolic health status. The BP mixture increased eFCR about 4 and 2.7 times on gross energy and crude protein basis, respectively, compared to CON, while other efficiency parameters, such as nitrogen efficiency or feed conversion efficiency, did not differ between treatments.

	Control	By-Products	SEM	P-Value
Total dry matter intake (kg)	21.2	21.1	0.4	0.825
NDF intake (g/cow and day)	7,691	8,148	158	0.019
Utilizable crude protein intake (g/cow and day)	3,127	3,140	56	0.841
Ether extract intake (g/cow and day)	639	819	15	<0.001
Starch intake (g/cow and day)	2,439	1,615	145	0.002
Energy intake (MJ NEL/cow and day)	140	138	3	0.440
Energy corrected milk yield (kg/day)	26.9	27.7	1.0	0.579
Nitrogen efficiency (milk N in % of N Intake)	24.1	24.1	0.4	0.993
Feed conversion efficiency (kg milk/kg dry matter intake)	1.28	1.28	0.02	0.998
Edible feed conversion ratio for energy (MJ/MJ edible input)	1.39	5.55	0.19	<0.001
Edible feed conversion ratio for protein (g/g edible input)	1.60	4.27	0.15	<0.001

Conclusions: The results of this study showed that by-products could substitute common concentrates with positive effects on the eFCR and without impairing performance. Dairy cattle can highly contribute to the net food production when the amount of potentially human edible feedstuffs is limited.

1. WILKINSON, J.M. (2011): Animal 5: 1011-1022.

*University of Natural Resources and Life Sciences, Vienna, Sustainable Agricultural Systems, Vienna

2. Milk yields and protein efficiency of dairy cows at restricted concentrate feeding

Milchleistung und Proteineffizienz von Milchkühen bei reduzierten Kraftfuttergaben

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The digestive functions of ruminants allow for high dietary proportions of roughage. It is discussed whether these proportions can be increased, aiming at reducing the demands for concentrates in livestock production (1, 2). On this background, an on-farm feeding trial was conducted to evaluate the influence of concentrate restrictions on milk yield and protein efficiency.

Methods: Twenty-three lactating cows (Swiss Fleckvieh) on an organic dairy farm in Berne, Switzerland were fed *ad libitum* with a total mixed ration (TMR) containing dry matter (DM) proportions of 0.32 grass silage, 0.30 maize silage, 0.21 hay, 0.09 alfalfa meal, 0.05 potatoes, and 0.03 soybean cake. Additionally the cows received on average 1.4 kg/d concentrate containing 24% crude protein (CP) and 1 kg/d concentrate containing 38% CP. Cows were kept in a stanchion barn which allowed individual feeding and weighing of the roughage intake. They were distributed to two groups (Conc+ [n=12]: fed as usual; Conc- [n=11]: fed with zero concentrates), balanced according to milk yield, milk protein content, and lactation stage. The experiment lasted for six weeks. In weeks 4-6, cows received extra hay in the morning. In weeks 3 and 6 feed intake was individually measured during four days; feed and individual faeces samples were drawn and analysed for nutrient concentrations and individual milk samples were taken for analysis twice weekly. Apparent CP digestibility was estimated based on the assumption that lignin would be totally indigestible. Data were analysed with a mixed model considering group as fixed and cow as random factor. For milk yield and composition, baseline values were included as covariable.

Results: Group Conc- compensated the omitted concentrates almost completely in terms of DM by a higher TMR and hay intake compared to Conc+. Intake of CP was not significantly reduced in Conc-. Although apparent CP digestibility was clearly lower in Conc-, milk yield was not significantly reduced and milk protein concentrations were similar in both groups. This led to identical protein efficiency ratios in both groups. Milk urea was lower in Conc- compared to Conc+.

Parameter	Group Conc+	Group Conc-	S.E.	P-value group
Concentrate intake [kg DM/d]	2.4	0	-	-
TMR + hay intake [kg DM/d]	17.8	21.1	1.53	<0.05
CP intake [kg/d]	3.24	2.95	0.028	0.197
Apparent CP digestibility [%]	68.6	60.8	0.01	<0.001
Milk yield [kg/d]	24.5	21.5	0.89	0.190
Milk protein [%]	3.15	3.31	0.010	0.474
Milk urea [mg/dl]	17.8	16.2	1.74	<0.05
Protein efficiency [g milk protein/g CP intake]	0.237	0.236	0.0002	0.989

Conclusion: Omission of concentrates was compensated by higher TMR intakes in terms of DM but not completely in terms of CP intake. However, the similar overall protein efficiency in both groups despite lower protein digestibility in Conc- suggests an increased endogenous protein utilisation of the cows with restricted concentrate feeding within the current study. The results indicate a potential to increase the utilisation efficiency of roughage CP in dairy cows with moderate milk yield levels if concentrates are restricted. Such results need to be confirmed with higher numbers of cows.

This study was supported by the Swiss Federal Office of Agriculture (BLW), Berne.

1) WILKINSON, J.M. 2011. Animal 5:1014-1022.
2) LEIBER, F. 2014. Organic Agriculture 4:269-273.

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3. Performance of lactating dairy cows fed different proportions of red clover silage in the diet

Leistung laktierender Milchkühe bei Fütterung verschiedener Rotkleesilageanteile in der Ration

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Question: In recent years, the interest for forage legumes as a source of home-grown protein has recurred. Due to the action of o-quinones formed by polyphenol oxidase, red clover does not undergo extensive proteolysis during ensiling, and protein degradation might be reduced in the rumen as well. Hence, it is conceivable that feeding red clover silage (RCS) in cattle nutrition enhances undegraded crude protein supply from forage, and consequently reduces the input of protein supplements such as soybean meal (SBM) or rapeseed meal. The aim of this study was to evaluate the effect of incremental replacement of maize silage (MS) and SBM with RCS and wheat on dry matter intake (DMI), milk production, and milk composition in dairy cows.

Methods: 44 primi- (n=18) and multiparous (n=26) Holstein-Friesian cows were used in a 4x4 Latin square design with 21-d periods. 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. Each period consisted of 13 d adaption and 8 d sample collection, consecutively. The RCS was prepared from the second growth of a pure red clover stand. Total mixed rations comprised of forage and concentrates (75:25), with ratios of RCS to MS in forage of 20:80 (diet RCS20), 40:60 (diet RCS40), 60:40 (diet RCS60), and 80:20 (diet RCS80) on a DM basis. The concentrates were based on lupine seed, SBM, and wheat. Within the concentrate, lupine seed was 35% (DM basis) for all diets, and wheat gradually replaced SBM with increasing proportion of RCS in the forage to obtain isonitrogenous diets. Thus, RCS20 contained 65% SBM (DM basis) in the concentrate, while RCS80 included no SBM. The DMI was daily recorded per group as the difference between the amount of feed offered and refused. Milk yield was recorded individually and milk samples were taken for analysis of milk composition. Mixed model procedure followed by ANOVA and Tukey tests and linear regression analyses were conducted.

Results: Intake of DM was lowest for cows fed RCS60 (P<0.001) and RCS80 (P<0.001). According to regression analyses, milk yield and protein content of milk linearly decreased with increasing proportions of RCS in the diet (P<0.001; P<0.001). No difference (P=0.655) was noted in milk lactose content among diets, while milk fat content was lower with RCS40 compared to RCS60 (P=0.020). Milk urea content was slightly lower with RCS60 compared to the other diets (P≤0.037). Yield of all milk components and ECM linearly declined as the proportion of RCS in the diet increased (P<0.001), as a result of differences in milk yield and partly in content of milk components.

	Treatment1					
Parameter	RCS20	RCS40	RCS60	RCS80	SEM	P-value
Dry matter intake (kg/d)	22.4c	21.5b	19.9a	20.0a	0.49	< 0.001
Milk (kg/d)	35.9c	34.9c	32.3b	30.2a	0.98	< 0.001
Energy corrected milk (kg/d)	35.3d	33.9c	31.6b	29.2a	0.74	< 0.001
Fat (%)	4.00ab	3.94a	4.04b	3.97ab	0.07	0.030
Protein (%)	3.20c	3.12b	3.09b	3.01a	0.06	< 0.001
Lactose (%)	4.82	4.81	4.81	4.81	0.02	0.655
Urea (mg/kg)	308b	303b	292a	303b	5.78	< 0.001

1RCS20, RCS40, RCS60, and RCS80=20%, 40%, 60%, and 80% (DM basis) red clover silage in forage, respectively. a,b,c,dmeans in the same row with different superscripts are significantly different (P≤0.05).

Conclusions: For an overall assessment of the use of RCS in dairy nutrition further information is needed on additional factors beyond the milk performance like costs of concentrates, RCS production, and nitrogen fertilizer or partitioning of dietary nitrogen.

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4. *In situ* ruminal crude protein and starch degradation characteristics of wheat grain

In situ-Abbau von Rohprotein und Stärke von Weizenkörnern im Pansen

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About half of the German wheat production is used as animal feed. In contrast, only about 8% of the cultivated genotypes are registered as fodder wheat, whereas at least 79% are listed according to the food quality classes A, B or E [1]. Therefore, many wheat genotypes are available for animal feeding differing in their nutritional characteristics. To optimize the utilization of wheat grains in diet formulation for ruminants, degradation characteristics of crude protein (CP) and starch (ST) in the rumen must be known. The objective of this study was to investigate the variation of *in situ* ruminal degradation characteristic and effective degradation (ED) of CP and ST of wheat grain by using genotypes that were selected to represent the quality classes and cover the variability of wheat grains.

Methods: The study used 20 wheat grain genotypes from the GrainUp consortium; growing, harvesting and storage conditions were the same for all genotypes. The samples contained per kg DM: 135±9 g CP, 21±2 g crude fat, 11.8±1 g aNDFom and 628±19 g ST. For *in situ* measurements samples were ground through a 2-mm screen and weighed into bags with 50 µm pore size. The bags were incubated for 0, 1, 2, 4, 8, 16, 24 and 48 h in the rumen of three lactating ruminally fistulated cows. At least 9 measurements (3 repetitions × 3 cows) were taken for each time and genotype. Grains and bag residues were analyzed for CP and ST content. Starch was analysed enzymatically. An exponential model [2] was fitted to the values of disappearance from the bags, with *a* (washout fraction), *b* (degradable fraction) and *c* (degradation rate) being the estimated parameters. Effective degradation (ED) of CP and ST was calculated from *in situ* estimates assuming passage rates *(k)* through the rumen of 5 and 8%/h as: $ED=a+[(b\times c)/(c+k)]$.

Results: Estimated CP and ST degradation parameters varied widely between wheat grain genotypes. The mean values for the *a*-fraction were 17% for CP and 36% for ST, and they ranged from 11 to 22% for CP and 25 to 49% for ST. The *b*-fraction averaged 81% for CP and 62% for ST with values ranging between 75 to 89% for CP and 51 to 74% for ST. The mean degradation rate (*c*) was lower ($P < 0.001$) for CP (21.3%/h) than for ST (65.2%/h) with variations from 17.8 to 27.2 (CP) and 38.4 to 99.2%/h (ST). The ED calculated for k=5%/h ranged between 80 and 85% for CP and 91 to 96% for ST with average values of 82 and 94% for CP and ST, respectively. Using a passage rate of 8%/h, variations between genotypes were slightly higher and average values were 76 and 91% for CP and ST, respectively.

Conclusions: The substantial variation in estimated degradation parameters *a* and *b* for both CP and ST was not reflected in the variation of the ED of the respective nutrients, due to the high degradation rate (*c*). Assumed passage rate also had only minor impact on the estimation of the ED of CP and ST for wheat grains. Therefore it is concluded that the variation in chemical and physical characteristics of different wheat grain genotypes has, if any, little impact on the ED in the rumen. Results indicate that it may be sufficient to consider differences in total CP and ST content between wheat grain genotypes and to assume average values for ruminal ED of CP and ST in diet formulation. As another consequence, consideration of specific feeding values for ruminants in registration of wheat varieties seems not to be necessary.

[1] Bundesministerium für Ernährung und Landwirtschaft (2013). Available from: http://berichte.bmelv-statistik.de, accessed on 06.11.2014.

[2] ØRSKOV, E.R., MCDONALD, I. (1979). J. Agric. Sci. 92: 499-503.

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5. Effects of two different alfalfa formulations (chaff vs pellets) on gastric mucosa in weanlings

Effekte unterschiedlicher Luzerne-Konfektionierungen (Häcksel vs. Pellets) auf die Magenschleimhaut bei Absetzfohlen

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The purpose of the study was to evaluate the effects of two different alfalfa formulations with different particle sizes (alfalfa chaff vs alfalfa pellets) on gastric mucosa in weanlings. We hypothesized that feeding a high proportion of fine particles provided by alfalfa pellets will impair gastric mucosa integrity whereas feeding long sized particles provided by alfalfa chaff might improve gastric mucosa health in weanlings.

Methods: 91 warmblood foals from one studfarm with a mean BW (± SD) of 256 ± 24 kg were included in the study. Immediately after weaning, foals were randomly allocated to one of four treatment groups. 1. Total mixed ration = control group (n = 21): In the control group a total mixed ration (TMR) was fed. The daily TMR for each foal was composed of 9 kg grass silage and 3 kg corn silage, 1.5 kg oats, 0.6 kg soybean meal, 120 g $CaCO_3$ and 40 g of a commercial trace element mixture. 2. Hay = hay group (n = 24): Foals were fed daily with 6 kg hay, 3 kg oats, 0.5 kg soybean meal, 120 g $CaCO_3$ and 40 g of a commercial trace element mixture. 3. Alfalfa chaff (n = 25): Foals were fed daily with 3 kg hay, 3 kg alfalfa chaff, 2.7 kg oats, 240 g soybean meal, 70 g $CaCO_3$ and 40 g of a commercial trace element mixture. 4. Alfalfa pellets (n = 21): In the alfalfa pellet group foals were fed daily with 3 kg hay, 3 kg alfalfa pellets, 2.7 kg oats and 40 g of a commercial trace element mixture. The amounts of feeds were standardized to supply an identical nutrient intake per day in each feeding group. In total, the different diets were fed for 18 days after the weaning process. Gastroscopy was performed immediately before weaning and after 18 days of the feeding period. Lesions were graded by using a validated scoring system (score 0: mucosa intact; score 1: areas of reddening or hyperkeratosis; score 2: small, single or multifocal lesions; score 3: extensive superficial lesions; score 4: extensive lesions with areas of apparent deep ulceration). The observers were blinded to the feeding of the foals. Stomach scores are presented as median and 25/75 % percentile. Wilcoxon signed rank test was used to compare the differences within a group before and after weaning. Kruskal-Wallis ANOVA was performed to compare the different treatment groups. Statistical significance was accepted at $p < 0.05$.

Results: Before weaning the prevalence of gastric mucosa lesions (≥ one lesion considering all locations in the stomach) was 89%, the lesions were mainly found in the Pars nonglandularis of the Curvatura major and Curvatua minor (data not shown). After weaning gastric mucosa lesions increased in part significantly in the different regions of the stomach. However, in the group fed alfalfa chaff or TMR weaning induced significant higher gastric mucosa lesions of the pylorus (Table). None of the foals showed any clinical signs commonly related to the presence of gastric mucosa lesions such as colic or slow eating.

Table: Grading of the stomach regions (score 0-4) after weaning in foals feeding different diets (results are expressed as median and the 25/27 % percentile is given in brackets)

Diet		Pars nonglandularis		Pars glandularis	
		Curvatura major	Curvatura minor	Curvatura major	Pylorus
TMR	n = 21	2 (1/3)[a]	2 (2/3)[a]	0 (0/0)[a]	0 (0/2)[a]
Hay	n = 24	0 (0/2)[a]	2 (1/2)[a]	0 (0/1)[a]	0 (0/0)[b]
Alfalfa chaff	n = 25	0 (0/1)[a]	2 (1/3)[a]	0 (0/0)[a]	1 (0/2)[a]
Alfalfa pellets	n = 21	2 (1/2)[a]	2 (0/3)[a]	0 (0/0)[a]	0 (0/0)[b]

Medians in the same column with unlike superscripts are different with $p < 0.05$

Conclusion: None of the different feeding regimes had a superior effect on gastric mucosa health in weanlings using the abrupt weaning process as a stress model to induce gastric mucosa lesions. In contrast to our hypothesis, higher pylorus lesions after weaning were observed for feeding alfalfa chaff and TMR but not for feeding alfalfa pellets or hay. The results were somewhat surprising as long alfalfa chaff is supposed to induce a more intensive chewing process than pelleted alfalfa hereby producing more buffering saliva. However, from the present study it can be speculated that alfalfa chaff might induce mechanical injury by passing the pylorus.

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6. A field study on the feeding of high performance show jumpers

Eine Feldstudie zur Fütterung von Hochleistungsspringpferden

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In this study the feeding of show jumping horses which started in the highest class of competition was investigated.

Methods: 51 horses from 12 stables were assessed by a standardized questionnaire on feeding, work and management answered by riders, owners and grooms. The daily rations were weighed. The body weight (BW) of horses was determined by measuring tape (1). The maintenance requirement of metabolisable energy (ME) was calculated by 0.52 MJ ME/$BW^{0.75}$. For work without rider per minute and kg BW of the horse for walk, trot and canter 0.17 kJ, 0.42 kJ and 1.8 kJ were added, for work with rider 0.19 kJ, 0.48 kJ and 1.92 kJ were added (2). Requirements and evaluations were taken from GfE 2014 (1). Data on composition of mixed feed were according to labeling and supplemental information from manufacturers, data on single feed from DLG-tables.

Results: The mean bodyweight of the show jumpers was 586 kg (470-690 kg), the mean body condition score was 5 (4 to 6/9). The training consisted of moving the horse in the walker, longing and riding. On average the horses walked 82 min/day, trotted 15 min/day and cantered 16 min/day. The amount of jumping in daily work was negligible. The mean ME intake was about 91 MJ and agreed well with the estimated mean for the energy requirements of 93 MJ ME which represents 1.5 times maintenance requirements. There was, however, considerable individual variation. Calcium requirements were met or exceeded. On average 3.3 times of calcium requirements were fed, the maximum intake was 5 times requirements, the calcium usually coming from high calcium concentrates and additional mineral supplements. The same was true for phosphorus. The Ca/P ratio was on average 1.8 and the minimum and maximum values of 1.1 and 2.8 in the required range, which is between 1.0 and 3.0. Potassium and chloride requirements were met by the roughage. Sodium intake ranged between 6 and 50 g (mean 20 g). The mean intake was in a similar range as the requirements of GfE (2014) for this level of work (25 g). Intake of the trace elements selenium, copper and zinc amounted to a mean of 1.8 mg (0.26-5.2), 145 mg (177-1536) and 611 mg (46-474), respectively. Vitamin A intake met or exceeded recommended daily allowance. It averaged 99666 IU/horse (24000-374721 IU) with a major percentage coming from added vitamin A in mixed feed and supplements. The same was true for vitamin D (mean intake per horse 11260 IE; 1401- 61580 IE). By contrast recommended intake of vitamin E was not met in all cases (mean intake 1087 mg/horse; 13-8107 mg). There was no clinical effect of over- or undersupply b of any nutrient. In 62 % of horses roughage provision did not balance energy requirements for maintenance. It is interesting to note that the horses which got enough roughage to meet their maintenance requirements were from the most successful stables. The hygienic quality of the feed was satisfactory in most cases. Conclusions: The present study showed that i) the ME-system of GfE (2014) works in a practical situation and ii) the feeding of top show jumpers can be considerably improved by nutrition consultation.

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7. Suitability of plantal *n*-alkanes for the estimation of dry matter intake and apparent digestibility in horses - pilot study

Eignung pflanzlicher kutikulärer n-Alkane zur Schätzung der Aufnahme und scheinbaren Verdaulichkeit von Futtertrockenmasse bei Pferden – Pilotstudie

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Feedstuffs for horses normally contain remarkable amounts of natural *n*-alkanes but their use as single markers seems to be unsuitable when the faeces quantity is unknown. Faecal dry matter (DM) output (DMO) has either to be determined from total collection or application of an artificial output marker. Diurnal defaecation is inconsistent and seem particularly to reflect intake dynamics. From this, we hypothesized that within specific time frames of a day it is possible to estimate DMO, and by use of plantal alkanes DM intake (DMI) and apparent DM digestibility (DMD), at a high level of accuracy. The aim of the study was to test this in horses with typical mealtime feeding of a hay-based diet.

Methods: Five adult horses received hay and small quantities of an oats-barley mix (1:1) trice a day in equal amounts. The animals were adapted to the test conditions for 5 days prior to the quantitative collection period. During 3 × 24 hrs, every defaecation was collected, a sample taken and prepared for gas-chromatographic analysis of natively occurring *n*-heptacosane (C27), *n*-nonacosane (C29), *n*-hentriacontane (C31) and *n*-tritriacontane (C33). DMD was determined from the measured DMI (mDMI) and faecal output (mDMO). Further, DMD was estimated (eDMD) from alkane concentrations in diets and faeces by consideration of spot samples of faeces selected to induce a minimal bias when compared with measured DMD (mDMD). The estimated DMI (eDMI) was obtained from eDMD and the estimated DMO (eDMO = averaged single defaecations of equal time frames × mean excretion frequency). The estimates and their measured counterparts were compared using wdifferences of least squares means with $P < 0.05$ as level of significance.

Results: Alkane recovery in faeces was determined to be 91 ± 11 (C27), 101 ± 9.76 (C29), 105 ± 8.73 (C31) and 98 ± 11% (C33). The mDMI, mDMO and mDMD were 12 ± 0.34 kg/d, 5.9 ± 0.30 kg/d, and 51 ± 2.3%, respectively. Defaecation patterns of DM and alkanes within and across days mainly reflected the intake characterized by mealtime feeding. Single meals probably led to excretion curves, which partly overlaid one another. Defaecation patterns decisively influenced the bias of eDMI, eDMO and eDMD. Specific minimal biased time frames were 0-2 (A), 2-4 (B) and 4-6 hrs (C) after morning feeding. For this, the first or only defaecation within the individual time frame was used for calculations. The estimates obtained for these time frames are shown in the table. These estimates were not significantly different from the measured ones. Later sampling did not allow coherent estimates. In general, C27 mostly underrated mDMI and mDMD, C31 predominantly overrated it.

Time frame	A	B	C
eDMI [kg/d]	12.8 ± 2.85 (C29)	11.6 ± 2.57 (C29)	12.5 ± 1.50 (C29)
	13.4 ± 3.28 (C31)	12.0 ± 2.48 (C31)	13.0 ± 1.60 (C31)
	12.6 ± 3.58 (C33)	11.6 ± 2.49 (C33)	12.7 ± 1.17 (C33)
eDMO [kg/d]	6.2 ± 0.95	5.5 ± 1.1	5.7 ± 0.60
eDMD [%]	51.1 ± 5.09 (C29)	52.4 ± 2.88 (C29)	
	49.5 ± 6.31 (C33)	52.4 ± 4.09 (C33)	

Conclusions: The presented approach may be useful to simplify experimental trials. However, excretion dynamics are probably influenced by ration type and gut peristaltic, where the latter one seems to be largely individual. It is suggested, that output fluctuations become lower when the feeding interval decreases and may be largely absent with *ad libitum* feeding. Then, any sampling time might be applicable. The approach needs to be validated with a larger quantity of horses and at different feeding regimes.

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8. Levels of precaecal digestible crude protein for various horse feedstuffs

Gehalte an praecaecal verdaulichem Rohprotein für verschiedene Pferdefuttermittel

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According to current recommendations (1) for protein horses rely entirely on the feed protein digested in the small intestine, whereas feed protein reaching the large intestine or protein synthesized in the large intestine do not contribute to amino acid supply of horses. The analytical approach for protein evaluation (1) is based on the determination of neutral-detergent-insoluble crude protein (NDICP) in feed. By using the corresponding proportion of ND-soluble CP (NDSCP), which is defined as potentially precaecal digestible, the precaecal digestible CP (pdCP) is estimated at 90 % of NDSCP. Only few data of pdCP in horse feed have been published. The aim of this study was to give an overview about variables concerning precaecal protein digestibility (pDCP) in forage and concentrate feedstuffs for horses.

Methods: The feed material consisted of 72 grass hay and 15 haylage samples from horse farms. In addition, 4 grass cobs, 19 oat grain, 15 barley grain, 4 soybean meal (SBM) and 2 rapeseed meal samples were analysed. The CP contents in hay and haylage were determined by near infrared spectroscopy, CP analysis of the remaining samples followed regulation (EC) no. 152/2009 III C. Determination of NDICP was conducted by CP fractionation according to (2). The NDSCP and pdCP were calculated using the recommendations of the GfE (1).

Results:The mean value of pdCP in grass hay was 46 g/kg dry matter (DM) with a remarkable range between 20 and 90 g/kg DM. Haylages, average 77 g/kg DM pdCP, had a higher concentration due to the higher initial CP contents and ranged from 45 to 116 g pdCP/kg DM. Regarding pDCP, mean values of hay and haylages were similar with 57% and 59%, respectively. Grain and protein feeds contained considerably more NDSCP and pdCP (99 g pdCP/kg DM for oat and barley grain and 340 g/kg DM for SBM) and had only little variation within the single variables compared to forage.

Table 1 Protein evaluation characteristics of hay and oat grain as the most important horse feedstuffs

	Grass hay (n=72)					Oat grain (n=19)				
Item	Mean	Min	Max	SD		Mean	Min	Max	SD	
CP (g/kg DM)	80	41	141	20		122	96	141	10	
NDICP (g/kg DM)	29	19	62	8		13	11	16	1.2	
NDSCP (g/kg DM)	51	22	100	15		110	84	130	10	
pdCP (g/kg DM)	46	20	90	14		99	75	117	9	
pDCP (%)	57	37	70	5.9		81	78	83	1.0	

Conclusion: The present study provides a first assessment of various feeds fed to horses concerning the new recommendations for protein supply. For ration planning and precision feeding, laboratory analyses are recommended due to the substantial range of pdCP values primarily in forage.

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2) Licitra, G., Hernandez, T. M., Van Soest P. J. (1996): Anim. Feed Sci. Technol. 57 (4): 347-358

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9. Effects of feed structure and protein source on performance and intestinal-immunological parameters of fattening pigs

Effekte der Futterstruktur auf die Leistung und intestinale, immunologische Parameter von Mastschweinen und Vergleiche zwischen konventionellem und heimischem Soja

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It is well known, that varying processing methods of feedstuffs can influence health and performance of fattening pigs. However, the knowledge about possible effects on immunological reactions of intestinal tract is limited, even though it takes a key function in the defense of potential harmful substances or antigens. Therefore the present study examines the relationships between feed structure and performance, gastric mucosal lesions and possibly related intestinal immunological reactions of T-cells of fattening pigs. Further, protein origin (conventional soybean meal vs. local protein sources) might interfere in immune modulation and was therefore included as additional factor.

Methods: Hundred crossbred barrows, individually housed, were divided into 4 feeding groups, which differed in feed structure (coarsely ground meal diet vs. finely ground, pelleted diet) and protein source (conventional soybean meal vs. local rapeseed meal/DDGS/soybean meal). Pigs were fattened about ten weeks by *ad libitum* feeding and slaughtered subsequently. Weights and feed intakes were recorded weekly. Alterations of stomach health were assessed by a modified macroscopic scoring system according to Betscher (2010)[1] (MSC 0 = normal - 4 = severe lesions). 13 animals per group were used for immunological investigations of intestine. Lymphocytes of jejunal tissue were isolated by using a modified method according to Solano-Aguilar et al. (2000)[3] and Davis et al. (2004)[2]. T-cell phenotyping was carried out by staining intestinal lymphocytes with monoclonal antibodies for CD3, CD4 and CD8 and flow cytometric measurements. For statistical analysis MIXED procedure of SAS Enterprise Guide 4.3 was used.

Results: Average daily gain (ADG) and daily feed intake (DFI) were significantly influenced by protein source ($p < 0.01$) while the highest ADG was achieved after feeding local protein sources. Coarsely ground feed tended to enhance ADG ($p=0.064$) and DFI ($p=0.074$). However, feed to gain ratio (FGR) was significantly decreased in groups fed pelleted diets ($p < 0.001$). Feeding of coarsely ground diets resulted in significantly lower MSC (MSC diet 1= 0.60; diet 3 = 0.68; diet 2=3.08; diet 4= 2.48; $p < 0.001$). Protein source also showed tendency effects on MSC ($p = 0.088$). Interestingly, feed structure showed significant effects on mean fluorescence intensity (MFI) of intraepithelial T-helper cells (CD4+) ($p=0.049$). Furthermore, it influenced also proportion of CD4+ and CD8+ of peyer´s patches and MFI of CD4+, respectively but not T-cells of lamina propria. Protein sources showed no significant influence on intestinal T-cells. However, T-cell proportion and MFI in lamina propria and peyer´s patches showed also alterations with variations in MSC (Table 1). No effects of different MSC were detected on intraepithelial lymphocytes or conditions of CD3+.

Stomach score	Intraepithelial lymphocytes [%]				Lymphocytes of lamina propria [%]				Lymphocytes of peyer´s patches			
	CD3	CD4	CD8	ratio CD4/CD8	CD3	CD4	CD8	ratio CD4/CD8	CD3	CD4	CD8	ratio CD4/CD8
Normal (0-0.5)	45.64	10.56	80.54	0.15	31.60	30.60	36.52	0.95	20.89	31.95	26.10	1.26
Middle (1-2.5)	41.02	6.28	80.75	0.08	28.30	21.79	33.13	0.66	18.90	25.45	22.50	1.16
High (3-4)	37.47	8.89	77.63	0.13	35.29	41.80	34.19	1.48	15.83	23.80	20.87	1.35
P-value	0.763	0.465	0.756	0.486	0.752	0.003	0.685	0.023	0.793	0.052	0.316	0.279

LSMeans; Kramer-Tukey; $p \leq 0.05$

Conclusion: Feed structure is an important factor which influences performance and stomach health. The increased FGR observed after feeding the coarsely ground meal might also be related to lower digestibility and an enhanced feed wasting.There are strong indications that the intestinal immune system is also influenced by feed structure. Local protein sources seemed to be superior to conventional soybean meal with regard to performance and stomach health.

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10. Morphometric traits of the Peyer's patch (as a sign of local immune response) in relation to different physical forms of one complete diet fed to growing pigs

Reaktionen der Peyerschen Platten als Zeichen einer lokalen Immunantwort wachsender Schweine auf eine unterschiedliche physikalische Struktur des Mischfutters

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The composition of the *digesta* displays an antigenic potential to stimulate the immune system of the host. The integrity of the mucosal barrier of the gastrointestinal tracts (GIT) as well as the composition of *digesta* (including gut microflora) were seen to be affected by the physical form of the diet[1,2,3,4]. It was hypothesized that the level of stimulation of the Peyer's patch (PP) in the terminal *ileum* (TI), considered as an immunological sensor[5] of the GIT, could be affected by the diets' structure. A feeding trial was therefore performed in order to screen the morphology and function of the PP in the growing pig.

Methods: A total of 32 growing pigs (initial BW: 8.30±0.83 kg) were individually housed in pens, without bedding material and fed a complete diet for one week (phase 1). The pigs were then switched to one of the four experimental diets (phase 2). These four diets were identical for botanical and chemical composition, each being offered to the pigs for 4 weeks: FP - Finely ground pelleted diet (dMEAN, 0.463 mm); CM - Coarsely ground meal diet (dMEAN, 0.880 mm); CP - Coarsely ground pelleted diet (dMEAN, 0.836); CE - Coarsely ground extruded (dMEAN, 0.659) diet. By the end of the experiment, all pigs were slaughtered and the last 3 cm of the TI were removed and fixed in buffered formaldehyde (2.5 % v/v), for embedding: all PP were analyzed according to the description given by Jung and co-workers[5] modified on this purpose[6]. The PP was analyzed for a) the height of the follicle associated epithelium (FAE, µm); b) germinal centers count (n GC/mm^2); c) parenchyma to connective tissue ratio (P/Ct). A global score (1 to 10-points scale) of the immune response of the PP was used to summarize all observed changes. A linear regression model was used for the statistical analysis of data, with the following formula, $Y_{ij} = \mu + D_i + W_j + D_i*W_j + e_{ij}$, where Y is the response of the PP (score), μ is the overall mean, D is the fixed effect of the diet (four levels according to the four dietary treatments), W is the fixed effect of the body weight (two levels, until and over 26 kg), $D*W$ is the interaction between the two fixed effects and e is the random residual.

Results: The PP showed a higher level of stimulation when the CM diet was offered to pigs (Table), followed by the CP diet from which, however, statistically differs (p=0.033), and more significantly from the CE diet (p=0.025) and the FP diet (p=0.011).

Table: Changes of the PP in young growing pigs related to the 4 physical forms of one diet.

Dietary treatments - ground - form	FP finely pellets	CM coarsely meal	CP coarsely pellets	CE coarsely extrudates
Animals, n	8	8	8	8
Parameters of the Peyer's patch				
FAE height (µm)	382±48.7	638±229	504±112	494±145
Density of GC (n/mm^2)	3.14±0.29	1.81±0.41	1.78±0.84	1.91±0.47
P/Ct Score (1-10)	1.77±0.03 8.03±0.69^b	1.65±0.03 4.16±0.78^a	1.63±0.04 6.67±0.78^{ab}	1.85±0.04 7.70±0.69^b

$^{a, b}$superscripts in a same row indicate a statistic significance for p

Conclusion: Different physical forms of one diet were associated with morphometric and functional changes in the overall evaluation of PP's immune response in young pigs. The level of stimulation of the PP allows to consider the role of the diet in determining diverse antigenic potentials of *digesta*, as a consequence of morphological adaptations of the mucosal barrier of the GIT, favouring the backflow contamination by hindgut microflora (different morphology of the ileocaecal valve).

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11. First results from laboratory studies about effects of added lactic acid bacteria and enzymes on fermentation of liquid feeds for pigs

Erste Ergebnisse über die Effekte von zugesetzten Milchsäurebakterien und Enzymen auf die Fermentation von Flüssigfuttermitteln für Schweine

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The fermentation of liquid feeds is a young method with increasing significance in pig production. In practice, various lactic acid bacteria (LAB) are partially used as starter cultures for fermentation of liquid feeds (1). Present study aimed to investigate the suitability of LAB and enzymes to optimize the fermentation process of liquid feed. The effects on decline of pH-value, the yield of short chain fatty acids and the quantity of undesired bacteria, yeasts and moulds were examined. In addition, it was investigated whether there were differences in amino acid reduction during the fermentation process and whether the fermentation could result in a higher availability of free phosphorus.

Methods: A constant quantity of substrate (typical ingredients for pig feed) were mixed with water (adjusted to 25% dry matter) and inoculated with a mixture of three homofermentative LAB (*L. paracasei*, *P. pentosaceus*, *L. rhamnosus* = Schaumalac Feed Protect) and a mixture of enzymes (amylase/pectinase). The fermentation (24h) was conducted under laboratory conditions at different temperatures (20°C, 30°C, 37°C). The pH-value and the yielded short chain fatty acids were recorded depending on temperature and time.

Results: The complex data as observed can be only partially reported here. The results show that both the fermentation temperature and the type of feed component have influenced the fermentation speed. The warmer the fermentation temperature and the higher the content of grain components in the substrate, the faster a decrease of the pH-value below 4 can be observed. Furthermore, the addition of LAB mixture led to a faster pH reduction and higher amount of short chain fatty acids. The added enzyme mixture did not affect the fermentation speed. In addition, the results of amino acid analysis led to conclusion that there was no reduction of the individual amino acids during the fermentation process, as demonstrated for lysine as example (Fig.). In opposite, a slightly increase of lysine content was observed for the addition of LAB mixture.

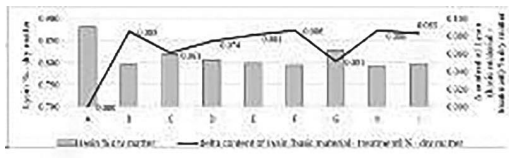

Figure: The concentration of lysine in dry matter before and after 24h fermentation depending on the test design; 1: basic material; 2: control; 3: LAB mixture; 4: enzyme mixture; 5: LAB mixture & enzyme mixture

Conclusion: The results of the present study show that the quickest reduction of the pH-value and cultivation of lactic acid occur at a fermentation temperature of 37°C. Furthermore, fermentation process seemed not to influence the content of native amino acids. In addition, the experiments have demonstrated that the selected LAB under study are very suitable for the aimed fermentation process. Thus, selected LAB should be used as starter cultures. Therefore, the current results provide important basic information.

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12. Formula compared to sow milk feeding changes morphology, barrier function and immune response in the small intestine of new-born piglets

Formulafütterung gegenüber Sauenmilch verändert Morphologie, Barrierefunktion und Immunantwort im Dünndarm von neugeborenen Ferkeln

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Question: Large litter sizes and low birth weight piglets in modern swine breeds have led to increasing attempts to develop artificial rearing programs with formula feeding. To date limited information is available about the impact of cow milk based formulas on the development of small intestinal physiology and immune response in new-born piglets. Since this may have long term impact on performance and susceptibility to disease later in life, we studied the effect of formula feeding on the development of intestinal barrier function and immune response in piglets.

Methods: Sixteen new-born piglets were either removed within 4h from their mothers, fed a formula based on skimmed milk powder and whey from cow milk (n = 8), or suckled by their mothers together with remaining littermates (n = 8). After two weeks, eight piglets per treatment were killed and jejunal tissues and ileal mesenteric lymph nodes (IL MLN) sampled. Jejunal morphometry (villus length, crypt depth, villus area) was determined in HE stained cross sections. Intraepithelial lymphocytes (IEL) and mast cells were counted. Lymphocyte populations in the jejunal epithelium and in the IL MLN were analysed via flow cytometry (n = 5-7). Epithelial chloride secretion upon stimulation with histamine and epithelial permeability towards macromolecules (horseradish peroxidase, HRP) were determined in Ussing chambers. Total mRNA was extracted from jejunal tissue and gene expression analysis was performed with cDNA transcripts for markers of epithelial cell turnover (caspase 3 and proliferating cell nuclear antigen, PCNA), pro-inflammatory cytokines (IL-8, TNF-a, IFN-y), mucin genes (MUC2, MUC4, MUC5AC), enzymes involved in histamine metabolism (diamine oxidase, DAO, histamine N-methyl transferase, HNMT) and glucagon-like peptide 1 and 2 receptors (GLP-1, GLP-2). Means were compared by ANOVA procedures. With regard to the unequal sample numbers a subsequent Tukey HSD test was carried out using SPSS (version 21.0, Chicago, USA).

Results: No significant differences in villus height were observed, whereas formula fed piglets had deeper crypts and bigger villus area ($P < 0.05$). Concomitantly, gene expression of caspase 3 and PCNA was higher ($P < 0.05$) in formula fed pigs. Epithelial permeability towards HRP (40,000 Da) was higher in formula fed piglets ($P < 0.05$). Secretory response towards histamine was higher in formula fed pigs whereas expression of genes involved in histamine bioconversion was not affected. The relative proportion of intraepithelial natural killer cells was higher in formula fed piglets ($P < 0.05$). In mesenteric lymph nodes, B cells were lower in piglets receiving formula. Pro-inflammatory cytokine gene expression (IL-8, TNF-a, IFN-y) was higher ($P < 0.05$) in jejunum of formula fed pigs.

Conclusions: Changes in cell turnover maybe related to bovine whey proteins such as lactoferrin in the formula, which have been shown to exert similar conditions in the gut of colostrum-deprived piglets (1,2). An initial impairment of the barrier function could be reasonable for the observed activation of the innate immune system in the formula fed piglets. However, lack of immunosuppression by sow milk factors appears also possible. Whether these adaptational changes affect the intestinal functionality and susceptibility to infectious diseases later in life need further elucidation.

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13. Effect of grinding intensity and hydrothermal processing of blue sweet lupin on apparent ileal digestibility of proximate nutrients and amino acids in pigs

Einfluss der Vermahlung und hydrothermischen Behandlung von blauen Süßlupinen auf die scheinbare ileale Rohnährstoff- und Aminosäureverdaulichkeit bei Schweinen

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Question: Blue sweet lupins (BSL) are considered as alternative protein source to soybean meal (SBM) in diets for growing pigs. However, their use may be limited due to high levels of non-starch polysaccharides (NSP) and other secondary (anti-nutritional) bioactive compounds (1). Mechanical processing including hydrothermal treatment may improve the nutritional value of BSL. The current study aimed at determining the influence of both grinding intensity and expanding of BSL on apparent ileal (AID) nutrient and standardized ileal digestibility of amino acid (SID AA) in pigs.

Methods: Four diets were formulated to meet the nutrient requirements of growing pigs (NRC 2012) and were based on soybean meal (21% in the diet) or differentially treated BSL (31% in the diet). Treatments of BSL were: ground to pass a 3 mm (CBL), 1 mm (FBL) sieve or ground to 1 mm and subsequently expanded (EBL) in a ring-cone expander (120°C). Twelve PIC x Danbred crossbreed pigs with an initial body weight of 20 kg were fitted with a simple T-cannula at the terminal ileum and fed the experimental diets (mash) in a 3 x 4 latin square design with an adaptation of 4 days followed by a 3 day cumulative digesta sampling time (sampling twice daily) for each experimental period. Titanium dioxide was used as digestibility marker. Proximate nutrients and minerals were determined using standard Weende procedures. AA were determined by ion exchange chromatography. The SID values for individual AA were calculated by correcting AID values for basal endogenous losses based on dry matter intake (2). Data were compared by ANOVA followed by LSD test using SPSS (version 21.0, Chicago, USA).

Results: Particle size distribution of final diets was similar between SBM and EBL diets whereas CBL and FBL diets had higher proportion of particles > 1.0 to ≤ 2.0 mm, lower proportions of > 0.15 to ≤ 1.0 mm, and FBL diets had higher proportions of particles < 0.15mm. Treatment increased the viscosity of the final diets slightly from 1.20 mPas (SBM) up to 3.04 mPas (EBL). Slight palatability issues were observed with EBL during the first sampling period but not thereafter. The AID of OM was higher ($P < 0.05$) in SBM compared to the other diets, and the AID of NfE was higher in SBM compared with FBL diets. The AID of EE was lower ($P < 0.05$) in CBL compared to the other diets. Finally, the AID of calcium was higher in CBL compared with EBL diets. No other significant differences in AID of proximate nutrient, calcium and phosphorus were observed. Surprisingly, the SID values for lysine, methionine, arginine and phenylalanine increased with treatment intensity (CBL < FBL < EBL). For example, SID of lysine increased from 73.7% (SBM) and 73.5% (CBL) to 75.4% (FBL) and 76.9% (EBL), respectively. On the other hand, SID values for alanine were lower in differently treated BSL diets compared to SBM diet.

Conclusions: The data are in good agreement with previous studies in pigs showing that hydrothermal treatment of BSL may improve the SID of AA, but has only minor effect on digestibility of crude nutrients, calcium and phosphorus (3). Processing (either fine grinding and/or subsequent expanding) of lupins may improve their nutritional value and justify at least partial replacement of SBM in diets for growing pigs.

1) Jezierny et al (2010): Anim Feed Sci Technol; 157; 111-128.
2) Jansman et al (2002): Anim Feed Sci Technol; 98; 49-60.
3) Yang et al (2007): Asian-Austral J Anim Sci; 20; 1229-1235.

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14. "Swine Protein Meal" (SPM) as a protein source in broiler diets: Effects on performance, composition of excreta and litter as well as on foot pad health

Untersuchungen zum Einsatz eines Nebenproduktes aus der Schweineschlachtung als Proteinquelle im Mischfutter für Masthähnchen: Effekte auf die Leistung, Exkremente- und Einstreuqualität sowie auf die Fußballengesundheit

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Introduction: It is well-known that Foot Pad Dermatitis (FPD) is a frequent disease in poultry, whereby the most important predisposing factor is the moisture content of excreta and litter[1]. Soybean meal (SBM) - the common protein source - often contributes to the development of wet litter due to its high contents of potassium and non-starch carbohydrates (e. g. stachyose, etc.). Therefore, different sources of protein can also contribute to an improvement of foot pad health.

The hypothesis of this study was, that reduced dietary SBM levels could improve the quality of excreta/litter and subsequently of foot pad health. The tested "alternative" protein source was a protein rich byproduct of pig slaughter (swine protein meal = SPM; 65 % crude protein, 19 % crude ash; up to now its use in food producing animals is not allowed in Germany), so that a special approval of the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) was obtained before the experiment started.

Methods: Two trials were performed with 100 male broilers (Ross 308) per trial. The 7 day-old birds were divided in four groups and fattened to 35 days of age. Feed and water were available continuously ad libitum and the intake was measured daily. Group 1 received a control diet without SPM, whereas the other diets contained 4, 8, and 12 % SPM, respectively. Hence, the amount of SBM could be reduced from 29.5 % (group 1) to 11.2 % (group 4). The external scoring of foot pads - acc. to Mayne et al. (2007[2], modified) - was repeated weekly. The measurements of the individual body weight (BW) and dry matter (DM) content of the litter (on group basis) were also conducted once a week. Statistical analyses were done by using the SAS software (analysis of variance and Wilcoxon-Two-Sample-Test, respectively, $p < 0.05$).

Results: The potassium contents in the diets decreased about 3 g/kg (group 1 vs. group 4). Increasing dietary levels of SPM led to lower feed intake and weight gains. Although the group with the highest amount of SPM in the diet had the highest DM content in the litter, the foot pad health was not influenced positively. Remarkable was the observed "stickiness" of excreta in groups with SPM in the diet.

	Group 1	Group 2	Group 3	Group 4
Diet composition				
Xp, g/kg (trial 1/trial 2)	205/192	212/199	216/205	224/213
K, g/kg (trial 1/trial 2)	8.43/8.59	6.99/7.63	6.20/6.05	5.25/5.34
Lys, g/kg (trial 1/trial 2)	12.4/11.5	12.2/11.6	11.5/11.7	11.8/12.0
MJ ME/kg (calc., trial 1/trial 2)	12.9/12.5	13.3/12.7	13.4/12.8	13.5/12.9
BW, d 35 (g), trial 1*	$2233^{a} \pm 154$	$2590^{b} \pm 231$	$2183^{a} \pm 358$	$1833^{c} \pm 281$
BW, d 35 (g), trial 2	$2592^{a} \pm 222$	$2454^{a} \pm 251$	$2255^{b} \pm 364$	$2100^{b} \pm 324$
DM content "final litter", %				
Trial 1 (n=1)	48.8	47.6	44.8	51.5
Trial 2 (n=1)	44.8	45.6	48.5	48.6
FPD-Score**, d 35 , trial 1	$1.74^{a} \pm 0.436$	$1.22^{b} \pm 0.410$	$2.04^{a} \pm 0.676$	$1.88^{a} \pm 0.576$
FPD-Score**, d 35 , trial 2	$2.36^{ab} \pm 0.902$	$1.96^{a} \pm 0.782$	$2.04^{ab} \pm 0.752$	$2.50^{b} \pm 0.866$

* Due to a technical failure, group 1 received a diet with a very low NaCl-content during the first days of trial. Therefore, results of the first trial are presumably influenced by this incident.

** low values indicate less "pathological" changes (0=healthy; 7=over half of the foot pad is necrotic)

Conclusion: The use of SPM in broiler diets seems to have an upper limit, not only because of the palatability of the diets, but also due to effects on the quality of excreta. In both trials, the usage of 8 and 12 % SPM in the diets resulted in higher (i. e. unfavourable) FPD-scores. As a combination of plant and animal protein sources seems to be advantageous for multiple reasons, a different composition of the diets (than used here) must be chosen for the application of higher amounts of SPM.

[1] *Kamphues et al. (2011): Übers. Tierernährg.,39, 147-195*

[2] *Mayne et al. (2007): Br. Poult. Sci.,4 (5), 538-545*

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15. Maintaining the activity of NSP-splitting enzymes during feed production: required for high litter quality and food pad health of broilers

Erhalt der Aktivität NSP-spaltender Enzyme im Mischfutter für Geflügel – eine Forderung im Interesse der Einstreuqualität und Fußballengesundheit

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Addition of enzymes (e.g. NSP-degrading ones) is common practice in feed production for poultry. But stability against different processing conditions like pressure, temperature, moisture is necessary to maintain the enzyme activity in the diet. The question occurs whether there are adverse effects of losses in enzyme activity on quality of excreta/litter and thus on food pad health.

Material and methods: In two experiments with 306/312 one day old broilers three consecutive feeding trials were conducted. Groups of 25/26 birds were housed in pens littered with wood shavings. The first 7-8 days of life birds were fed a commercial starter diet and following experimental diets were fed until slaughter. Those composed on the same "basal mixture" (wheat, soybeanmeal, soyoil, vitamin and mineral mixture including i.a. xylanase and glucanase, calciumcarbonat; ~13 MJ ME, 240 g/kg XP as fed) and only differed in the grinding and compaction, as a finely ground and pelleted diet (PF), a coarsely ground and pelleted diet (PC), a pelleted diet with 22 % of whole wheat added to finely ground components prior to pelleting (PW) or a coarsely ground and expanded diet (EC). Due to the processing conditions the enzyme activity at least for the xylanase was reduced (Table 1).

Table 1:	PF	PC	PW	EC
Mill type/compaction	hammer/pellet	roller/pellet	hammer, unground/pellet	roller/extrudate
Temp. (Exp. 1/2; °C): - conditioning	60 / 66	60.5 / 67	62.5 / 67	66.5 / -
- granulation	83.5 / 81	81 / 80	83 / 82	87 / 86
- extruders head	- / -	- / -	- / -	105 / 110-130
Xylanase activity (Exp. 1/2)1	800 / 776	550 / 593	617 / 898	163 / 0

[1]visco units/kg diet measured in Exp 1, trial 1+2 and Exp. 2, trial 2+3; before pelleting/extrusion (max.-min.): 865-1444 visco units/kg diet

Food pad health (according to Mayne et al. 2007) and moisture content of the whole litter of each group/pen were analyzed at the end of each trial (except the first trial of the second experiment). The foot pad scores were evaluated by using the mean of both feet. The data were analysed using the GLM procedure of the SAS Institute Inc. (2005) software.

Results, discussion and conclusion: Following the average dry matter content of the final litter and the mean foot pad scores measured on day 35/36 of life are presented (Table 2).

Table 2:	PF	PC	PW	EC
Final litter, DM (%)*	46.7 / 39.5	42.5 / 47.2	42.6 / 42.9	39.4 / 35.7
Foot pad scores: Experiment 1	2.98c ± 1.11 (n=43)	3.77b ± 0.71 (n=47)	3.71b ± 0.93 (n=46)	4.53a ± 1.22 (n=47)
Foot pad scores: Experiment 2	3.98b ± 0.75 (n=42)	2.95c ± 1.02 (n=39)	3.61b ± 0.88 (n=40)	4.79a ± 1.09 (n=42)

*Measured in Exp.1 trial 1, 2, 3 and in Exp. 2 trial 2, 3; [abc] denote significant differences in the same row ($p<0.05$)

By calculating the mean water input (drinking water + water from the diet), water output (excreta + final litter + water of carcasses) as well as the difference between those values (representing the water disappearing via air by drying of litter) group EC had in general lowest amounts disappearing via air. Presumably the losses in xylanase activity changed the litter quality and the water binding and/or drying properties of excreta/litter. Due to unfavorable conditions in feed production the enzyme activity may be lost with possibly adverse impact on litter quality. When suddenly a poor litter quality and Foot Pad Dermatitis occur, besides well-known parameters like the electrolyte or the protein content of the diet or effects of ingredients like triticale the enzyme activity should be taken into consideration. To avoid risks associated with lowered enzyme activity the post pelleting application of NSP-splitting enzymes is recommended and widely used.

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16. Influence of different maize genotypes in diets without and with added phytase on precaecal $InsP_6$ hydrolysis and P digestibility in turkeys

Einfluss verschiedener Maisgenotypen in Rationen mit und ohne Phytasezusatz auf die Hydrolyse von InsP6 und die Phosphorverdaulichkeit bis zum Ende des Ileums von Puten

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Maize is one of the main ingredients used in turkey diets. Different genotypes of maize vary in their content of *myo*-inositol (1,2,3,4,5,6) hexakis dihydrogenphosphate ($InsP_6$), the primary storage form of P in maize. Intestinal hydrolysis of $InsP_6$ from different maize genotypes was so far not studied in turkeys. Hence, this study investigated potential differences in precaecal $InsP_6$ hydrolysis and P digestibility in turkeys fed diets containing different maize genotypes.

Methods: Four maize genotypes differing in their concentrations of $InsP_6$-P (1.9-3.1 g/kg DM) and total P (2.6-3.6 g/kg DM) were added to a low-P basal diet (**BD 1**, 3.0 g P/kg DM) at the expense of maize starch (43 %). The P content of these four genotype diets (**GD 2-5)** ranged between 4.1 and 4.8 g/kg DM and the $InsP_6$-P content was of 2.4 (GD 2-3) and 2.7 g/kg DM (GD 4-5). The Ca:P ratio was adjusted to 1.6:1 in all diets. Each diet was either supplemented with 0 or 500 FTU per kg diet of an *E. coli* 6-phytase (**Phy**, Quantum™ Blue). TiO_2 was used as indigestible marker and diets were fed as pellets. On day 20, turkeys were randomly distributed to 8 incomplete blocks of 8 pens and 10 treatments (15 birds per pen). This resulted in 8 and 6 replicates for BD and GD, respectively. On day 28, digesta was collected from the terminal half of the ileum, pooled on a pen-basis, freeze-dried and analysed for $InsP_6$, P and TiO_2. $InsP_6$ was determined by HPIC and UV detection at 290 nm following postcolumn derivatisation using a Dionex ICS-300 system. P and TiO_2 were analysed by using ICP-OES after acid hydrolysis. A mixed model was used for statistical analysis containing 'Diet type' (BD or GD), 'Phy' (0 or 500 FTU), 'Diet' (1-5) and their interactions as fixed effects and the incomplete block as random effect (SAS 9.3).

Table: Precaecal $InsP_6$ hydrolysis and P digestibility (%) in turkeys (means)

	$InsP_6$ hydrolysis		P digestibility	
Phy (FTU/kg)	0	500	0	500
Diet				
BD 1	26^{b}	37^{a}	37^{c}	46^{a}
GD 2	15^{c}	32^{a}	28^{d}	37^{c}
GD 3	6^{d}	34^{a}	22^{e}	39^{bc}
GD 4	7^{d}	33^{a}	23^{e}	38^{bc}
GD 5	7^{d}	38^{a}	25^{de}	42^{b}
Pooled SE	2.30		1.33	
P-value (ANOVA)				
Diet type	<0.001		<0.001	
Phy	<0.001		<0.001	
Diet	0.275		0.148	
Diet type × Phy	<0.001		0.010	
Diet × Phy	0.029		0.009	

$^{a-c}$ Different superscripts indicate significant differences within trait ($P \leq 0.05$)

Results: Overall levels of $InsP_6$ hydrolysis and P digestibility were low (Table). No significant effect of the 'Diet' was detected. However, a significant interaction was observed for 'Phy' and 'Diet' as well as for 'Phy' and 'Diet type'. Without 'Phy' addition $InsP_6$ hydrolysis and P digestibility were higher in BD 1 and GD 2 compared to GD 3, 4 and 5 whereas supplementation of 'Phy' led to a similar $InsP_6$ hydrolysis and less differences in P digestibility particularly for BD 1 and GD 3, 4 and 5. 'Phy' supplementation increased $InsP_6$ hydrolysis and P digestibility in all diets.

Conclusions: Differences in $InsP_6$ hydrolysis between maize genotypes explained differences in P digestibility. Phy supplementation may compensate for such differences. However, irrespective of Phy supplementation $InsP_6$ hydrolysis and P digestibility were low in turkeys compared to broilers fed with similar diets containing the same genotypes [1]. Whether this is attributed to a lower intestinal and/or microbial phytase activity in the gastrointestinal tract of turkeys compared to broilers needs further investigation

[1] INGELMANN et al. (2013): 125. VDLUFA-Kongress, Kurzfassungen und Referate, 122.

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17. First applications of novel protein hydrolysates from animal by-products as fish meal substitute in fish diets - effect on growth performance and protein utilization in Nile tilapia

Die Eignung neuartiger Proteinhydrolysate aus tierischen Nebenprodukten als Fischmehlalternative im Fischfutter – Einfluss auf Wachstumsleistung und Proteinverwertung bei Nil Tilapia

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Fish feed formulae relying less on fishmeal (FM) requires novel substitutes and accurate information about the replacement value of new protein sources. The aim of the current study was to evaluate the potential of novel protein hydrolysates from animal by-products as FM substitute in Tilapia diets with respect to growth performance and protein/amino acid utilization.

Methods: A growth experiment was conducted with 320 juvenile all-male Nile tilapia (*O. niloticus*) making use of a control diet (18% FM) and four experimental diets where 15% of FM was replaced by protein hydrolysates originating either from rendered pork (AniPor) or poultry (AniPou) material. Each type of hydrolysate was provided (ANiMOX GmbH, Berlin) by two different treatments (autoclaving at 130°C resp. 160°C). Diets were formulated to be similar both in crude protein (CP) and energy content. Essential amino acid supply of the diets was within the recommendations for Tilapia, except methionine (Met) for further investigation of Met efficiency. Growth response and protein/amino acid utilization were studied in a semi-closed in-door water recirculation system with 20 tanks (320 l/tank; water temperature 26.9±0.3°C; regulated photoperiod 12 h light/12 h dark). Four replicate groups per diet (16 fish per tank) were utilized in a 56 d growth experiment with feeding until apparent satiation twice daily. Ten fish at the beginning and five fish per tank at the end of the growth study were analyzed for body composition to calculate N-deposition and N-excretion for evaluation of protein/amino acid utilization. Statistical analyses (ANOVA, Tukey-test) were conducted by R-software (version 3.0.2).

Results: In general, feed intake (FI) and growth performance following the control diet were superior to the diets with novel protein sources. Nevertheless, regarding FI and final body weight (BW) no significant effect was observed between diets containing the protein hydrolysates. However, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were significant inferior in the diet with AniPor 160, indicating a treatment induced protein damage and lower protein quality of this protein source for tilapia. The final analyses both of protein utilization and Met efficiency data are still in progress.

Diet	Control	AniPor 130	AniPor 160	AniPou 130	AniPou 160
CP [% dry diet]	43.9	44.9	44.7	44.8	44.6
Met [% dry diet][2]	0.61	0.54	0.53	0.56	0.57
TSAA [% dry diet][2]	1.17	1.07	1.06	1.09	1.10
BW_{start}/fish [g]	24.6 ± 0.8[1]	24.8 ± 0.8	25.5 ± 2.1	25.0 ± 1.5	24.1 ± 1.7
BW_{final}/fish [g]	109.9^{a} ± 13.3	68.4^{b} ± 12.2	54.1^{b} ± 22.5	61.8^{b} ± 10.8	59.3^{b} ± 11.8
FI [g dry diet/$kg^{0.67}$/d]	10.3^{a} ± 0.6	7.3^{b} ± 0.9	5.9^{b} ± 1.4	6.9^{b} ± 0.8	6.6^{b} ± 0.9
SGR [%]	5.33^{a} ± 0.51	3.58^{b} ± 0.53	2.48^{c} ± 1.16	3.20^{bc} ± 0.43	3.17^{bc} ± 0.48
FCR [g/g]	1.22^{a} ± 0.07	1.43^{a} ± 0.08	1.91^{b} ± 0.64	1.53^{ab} ± 0.09	1.49^{ab} ± 0.12
PER [g/g]	2.05^{a} ± 0.11	1.84^{ac} ± 0.11	1.49^{b} ± 0.45	1.72^{bc} ± 0.11	1.78^{bc} ± 0.14

[1] standard error; [a,b] different superscript letters reveal significant differences between diets ($p<0.05$);
[2] recommended (1) levels [% dry diet]: 0.70 Met; 1.00 Total sulphur containing amino acids (TSAA)

Conclusion: Partial replacement of FM by protein hydrolysates originating from rendered pork or poultry by-products appears promising, but further research is needed to optimize the treatment conditions for both protein output and minimized damage of the protein fraction.

(1) NRC (National Research Council), 2011. Nutrient requirements of fish and shrimp.

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18. Protein quality assessment of novel protein sources from thermal hydrolysed animal by-products in the laboratory rat

Proteinqualitätsbewertung neuer Proteinquellen aus thermisch hydrolysierten tierischen Nebenprodukten bei der Laborratte

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Protein hydrolysates from animal by-products may contribute to improved sustainability of feed proteins in the future. The current study was conducted to evaluate the acceptance and protein quality of various types of hydrolysed animal by-products for the laboratory rat as a model animal. In addition, the experiments should indicate if the applied processing conditions are a damaging factor (Maillard reaction, reduced amino acid (AA) availability, AA degradation etc.) for the yielded dietary protein quality and if the treatment conditions have to be modified.

Methods: The assessed animal protein hydrolysates originated either from rendered pork (AniPor) or poultry (AniPou) by-products (Category 3). The thermal hydrolysis process (ANiMOX GmbH, Berlin) at graded temperatures (130°C resp. 160°C) was assigned to achieve high protein yields during processing. AA composition of the protein sources was analysed, but reported elsewhere (1). Isonitrogenous and isoenergetic diets were formulated to contain either 15% protein hydrolysate (test diets) or 9.25% casein as reference protein (Control). Further ingredients were barley, wheat, wheat starch and soybean oil. The N balance study (6d collecting period) utilized 35 male Wistar rats for assessing N-balance data from 2 consecutive collecting periods, true N-digestibility (TD), biological value (BV) and standardised net protein utilization (NPU_{std}). The model parameter daily N maintenance requirement (128mg/$BW_{kg}^{0.67}$) and theoretical maximum of daily N-deposition (1112 mg/$BW_{kg}^{0.67}$) were applied to standardise NI for NPU_{std} at 1300 mg/$BW_{kg}^{0.67}$ according to (2). Statistical analyses run with one-way ANOVA using SPSS 21.0.

Results: Diets containing the novel protein sources were accepted by the rat. TD of the protein fraction was generally lower than in the reference protein casein and significantly lower following treatment 160°C both in AniPor and in AniPou. But the results of BV indicate that the post-absorptive utilization of the hydrolysed proteins at 160°C was not impaired, AniPor 160 yielded the highest BV between the hydrolysed proteins. Finally, NPU_{1300} indicated that mild treatments yielded superior protein quality. However, this effect was only significant with AniPou from poultry by-products. The protein quality of the reference diet was not achieved by any of the other protein sources.

Diet	AniPor130 (n=13)	AniPor160 (n=14)	AniPou130 (n=13)	AniPou160 (n=14)	Casein (n=14)
MBW[1)]	126.6 ± 11.9	123.5 ± 8.6	126.8 ± 11.2	124.2 ± 10.0	132.9 ± 11.1
DMI[2)]	11.7 ± 1.1	11.9 ± 1.0	11.8 ± 1.0	12.0 ± 1.0	11.1 ± 0.6
NI[3)]	1314^{ab} ± 70	1364^{b} ± 57	1346^{b} ± 46	1357^{b} ± 66	1270^{a} ± 76
NB[4)]	517^{ab} ± 39	492^{a} ± 43	574^{b} ± 71	460^{a} ± 103	719^{c} ± 83
TD(%)[5)]	88.8^{b} ±1.6	70.9^{a} ± 6.3	86.8^{b} ± 4.5	73.0^{a} ± 5.2	95.0^{c} ± 1.0
BV(%)[6)]	55.4^{a} ±4.6	64.4^{bc} ± 5.5	60.2^{ab} ± 6.8	59.9^{ab} ± 11.8	70.2^{c} ± 4.5
NPU(%)[7)]	49.3^{ab} ± 3.4	46.2^{a} ± 3.7	52.9^{b} ± 5.4	44.0^{a} ± 7.5	65.9^{c} ± 5.2

Means (SD) [1)] MBW = mean BW (g); [2)] DMI = DM-intake (g/d); [3)] NI = daily N-intake (mg/$BW_{kg}^{0.67}$); [4)] NB = daily N-balance (mg/$BW_{kg}^{0.67}$); [5)] TD = true N-digestibility; [6)] Biological value; [7)] Net protein utilization, NI standardised at 1300 mg/$BW_{kg}^{0.67}$; different superscript letters reveal significant differences between diets (p

Conclusion: Mixed diets containing hydrolysed proteins from animal by-products were generally accepted by rats. Treatment conditions during processing are an important factor for the provided protein quality, but currently it is not clear whether the resulting effects on observed protein value are associated with lower absorption rate or higher endogenous N-secretion.

1) SÜNDER, A, HÖHLING, A and LIEBERT, F (2015): Proc. Soc. Nutr. Physiol. 24.

2) PASTOR, A, WECKE, C and LIEBERT, F (2012): Proc. Soc. Nutr. Physiol. 21.

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19. Substitution of common concentrates with by-products: Effects on ruminal fermentation, nutrient degradation, and microbiota *in vitro* (Rusitec)

Substitution eines Standardkraftfutters durch Nebenprodukte: Auswirkungen auf Pansenfermentation, Nährstoffabbau und Mikrobiota in vitro (Rusitec)

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Question: Due to economic advantages, high availability, as well as low animal feed vs. human food competition, by-products are important feedstuffs in dairy nutrition and their role will become more important in the future. In our previous in vivo trial, we tested the effects of substituting common concentrates (CON) with a mixture of by-products (BP) on milk performance, nutrient intakes, blood parameters and the edible feed conversion ratio. The aim of this study was to further investigate the effects of this substitution on ruminal fermentation, nutrient degradation, and the abundance of key microbiota in the rumen.

Methods: The experiment consisted of 2 experimental runs, comprising 12 fermentation vessels each time and lasting for 10 days, with the last 5 days serving for data collection. It was a 2 x 2 factorial arrangement with 2 concentrate types (CON and BP) and 2 concentrate levels (25 and 50% based on dry matter). The composition of CON was 27% peas, 20% maize, 20% field beans, 16% oats, 13.5% wheat, 3% molasses, and 0.5% commercial mineral and vitamin mixture. The composition of BP was 41.5% maize middlings, 30.5% beet pulp, 15.5% rapeseed cake, 9% soya cake, 3% molasses, and 0.5% commercial mineral and vitamin mixture. BP diets had higher fat and NDF contents, whereas starch content was lower compared to CON. The forage mixture consisted of grass silage and alfalfa hay in a ratio of 3 : 1 on dry matter basis. Mean values of the last 5 days of each experimental run were subjected to ANOVA. Concentrate level and type, as well as their two-way interactions were included as fixed effects, whereas fermenter and run were considered as random effects.

Results: NDF and NFC disappearance were higher for BP diets, but disappearance of CP decreased. Total CH_4 and CO_2 formation did not differ between concentrate types, but formation of CH_4 and CO_2 per unit NDF degraded was lower for BP, with a more prominent effect at the higher concentrate level. Concentrations of propionate in the fermenter fluid were elevated for the BP mixture, resulting in a lower acetate-to-propionate ratio, whereas butyrate and caproate were less present compared to CON. The BP mixture increased abundance of bacteria of the genus *Prevotella*, again with a more distinctive effect when fed at 50% inclusion rate, while presence of bacteria, protozoa, methanogens and anaerobe fungi was not affected by the concentrate type.

Concentrate level (%)	25		50			P-Value		
Concentrate type	CON	BP	CON	BP	SEM	Type	Level	Interaction
CH_4 (mL/g $NDF_{degraded}$)	11.8	9.2	19.1	11.2	1.43	<0.001	<0.001	0.033
CO_2 (mL/g $NDF_{degraded}$)	64.8	49.4	115.3	68.0	3.29	<0.001	<0.001	0.024
Organic matter disappearance (%)	58.5	58.4	63.9	61.7	0.6	0.057	<0.001	0.079
CP disappearance (%)	69.7	67.5	73.7	67.1	0.8	<0.001	0.015	0.005
NDF disappearance (%)	25.2	30.0	22.8	29.5	0.9	<0.001	0.095	0.270
Total volatile fatty acids (mmol/L)	104.4	103.8	112.9	108.1	2.1	0.215	0.006	0.323
Acetate (% of total)	53.2	52.6	53.8	53.2	1.5	0.325	0.388	0.993
Propionate (% of total)	20.7	23.2	19.6	22.2	2.6	0.002	0.144	0.976
Butyrate (% of total)	12.1	11.0	12.9	11.3	0.4	0.002	0.170	0.490
Bacteria (log_{10} gene copies/mL)	9.12	9.15	9.15	9.14	0.08	0.681	0.923	0.563
Protozoa (log_{10} gene copies/mL)	6.78	6.49	7.10	6.92	0.13	0.109	0.002	0.550
Genus Prevotella (% of bacteria)	10.29	12.20	11.44	16.19	2.16	<0.001	0.002	0.034

Conclusions: Despite higher fiber and lower starch contents, BP increased abundance of bacteria belonging to genus *Prevotella* and increased concentrations of the glycogenic volatile fatty acid propionate, which is important to high yielding dairy cows. The results of this study indicate that these by-products have the potential to adequately substitute common concentrates.

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20. Does pressure affect *in vitro* rumen fermentation? A comparison of pressure-release systems for *in vitro* incubation of feed substrates

Hat Druck einen Einfluss auf die in vitro Pansenfermentation? Ein Vergleich zwischen drei Druckentlastungssystemen bei der in vitro Inkubation von Futtermitteln

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The Ankom RF Gas Production System measures the fermentation kinetics by continuously recording gas production by means of pressure sensor modules. These modules also release the gas at a constant rate, thereby maintaining a similar pressure within flasks throughout incubation. A previous study has mentioned that pressure might alter microbial fermentation in *in vitro* systems (1). Therefore, the aim was to determine if there are differences in the *in vitro* fermentation when incubation flasks are closed with the Ankom RF module (Ankom), with a syphon-type lid (Syphon), or with no release of pressure (Closed) over a 24-h-incubation period.

Methods: Two grams of a hay:concentrate mixture (70:30; on fresh matter basis) were weighed into 500-ml-flasks in three replicates per system and 300 ml of rumen fluid mixed with bicarbonate buffer (2:1 ratio) were added prior the start of the incubation. All flasks were continuously flushed with CO_2 until sealing and incubated in a water bath at 39°C. Rumen fluid was collected before the morning feeding from two rumen fistulated Jersey cattle fed on grass silage ad libitum and supplemented with a concentrate mixture (2 kg/d). The experiment was repeated in three separate runs. After 24 h of incubation, the whole content of each flask was centrifuged at 500 *g* for 10 min at 4°C to obtain a fraction of non-degraded feed particles and solid-associated microbes (Feed+SAM). The remaining supernatant was centrifuged again at 20,000 *g* for 8 min at 4°C to separate liquid-associated microbes (LAM). The nitrogen (N) and purine bases (PB; = adenine + guanine) contents in Feed+SAM and LAM pellets were determined by Kjeldahl and high-performance liquid chromatography, respectively. Additionally, a sample of the final supernatant was taken for volatile fatty acids (VFA) and ammonia-nitrogen (NH_3-N) analyses. Differences between systems were tested by an analysis of variance using the GLM procedure of SAS (Version 9.4).

Results: There were no differences in total VFA and NH_3 concentrations as well as the proportions of individual VFA between the three systems (Table 1). Moreover, the contents of N and PB were similar in both, Feed+SAM and LAM.

Table 1. Concentrations of ammonia-nitrogen (NH_3-N) and total volatile fatty acids (VFA) in liquid phase, and of nitrogen (N) and purine bases (PB) in microbial pellets after 24 h of incubation in three pressure release systems (Means ± standard deviation) (n = 9).

Parameters	Sealing system			P-value
	Ankom	Syphon	Closed	
NH_3-N (mg/ml)	0.21 ± 0.049	0.22 ± 0.034	0.21 ± 0.032	0.41
VFA (Mol/ml)	50.4 ± 14.19	52.5 ± 13.03	51.1 ± 11.81	0.82
N (mg/100 mg DM)				
Feed+SAM	4.2 ± 1.11	4.2 ± 0.99	4.1 ± 1.09	0.60
LAM	6.2 ± 0.50	6.3 ± 0.50	6.5 ± 0.29	0.36
PB (µMol/100 mg DM)				
Feed+SAM	1.18 ± 0.202	1.19 ± 0.157	1.14 ± 0.207	0.62
LAM	2.36 ± 0.433	2.45 ±0. 280	2.57 ± 0.401	0.15

LAM, liquid-associated microbes; SAM, solid-associated microbes.

Conclusions: Although pressure in closed flasks was around 965 mbar after 24 h and thus much higher than in Ankom (4.8 mbar) or Syphon (0.7 mbar) systems, no negative effects were observed in studied parameters. Hence, in 24-h-incubations of approximately 2 g feed material, closed flasks or those with a syphon can be used without differences in the fermentation pattern of the substrate.

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21. Investigation of changes in the bacterial microbiom in the large intestine of calves fed with pectin containing milk replacer

Untersuchung zur Veränderungen des bakteriellen Mikrobioms im Dickdarm von Kälbern nach Fütterung eines pektinhaltigen Milchaustauschers

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Pectins are supposed to have beneficial effects on the intestinal health (1) and may prevent diarrhoea during rearing. They were fermented in the hindgut and probably alter the microbial community there. This may promote the gut's health *inter alia* by butyrate production. In previous *in vitro* studies with calves' faeces particularly highly methylated citrus pectin (HMCP) revealed such favourable effects (2). The aim was to investigate the impact of HMCP as ingredient of a milk replacer (MR) for calves on the bacterial faecal microbiom being a rough proxy of the conditions in the hindgut.

Methods: 16 female Holstein-Friesian calves were randomly divided into control and treatment group (CG and TG) at d4 of life. CG calves received a commercial MR on the basis of skim milk powder and sweet whey powder (SWP) and TG calves the MR with 1.2% of HMCP in the solid phase in exchange for SWP. Until d14 of life calves were individually housed in calf hutches and 3 times/d 3 L of liquid MR were offered (200 g MR/L water). Thereafter, they were held in groups and MR was slowly reduced to 2 L/d until d63. Further, calves received a total mixed feed and hay *ad libitum*. At d3, d14 and d63 of life faeces was sampled and bacterial DNA extracted (innuSPEED Stool DNA Kit, Analytik Jena, Jena, Germany). Specific fragments were amplified with universal primers S-D Bact-0968-a-S-GC (forward) and S-D-BAct-1401-a-A-17 (reverse; 3). Amplificates were verified with electrophoreses and differentiated with denaturing gradient gel electrophoreses. Dominating bands were cut out with sterile scalpels, re-amplified, purified and sequenced. Polyacrylamid gels were evaluated with Bionumerics version 5.0 (Applied Maths, Inc., Sint-Martens-Latem, Belgium). The band patterns of different lanes, each lane represent a sample of one calf belonging to CG or TG respectively, were verified with Dice similarity coefficient depending on sampling day (d3, d14, d63) and sequences compared with the database of the National Center for Biotechnology Information.

Results: DNA was extracted and amplified from all samples and 125 bands were assigned to a bacteria species. The comparison of all 6 polyacrylamid gels with DSC displays a high similarity in the allocation of bands between CG and TG for d3 and d63. At d14 a homogeneous pattern of bands was identified for CG, but not for TG. In both groups and all samples *Clostridium perfringens* and *Escherichia coli* dominated the bacterial microbiome at d3. Bacteria species with supposed beneficial effects, like *Faecalibacterium prausnitzii* (TG) and *Lactobacillus* sp. (CG, TG), were detected in the samples from d14 of life. But non-pathogenic bacteria species such as *Ruminococcus* (R.) *gnavus* and *Bifidobacterium longum* dominated at that time and were replaced by *R. faecis* and *Butyrivibrio* sp. after 9 weeks.

Conclusions: Whereas the 3d old calves had a similar initial situation regarding their faecal microbiome, the HMCP-enriched MR only enhanced the heterogeneity of the bacterial microbiome until d14 of life, which was as a transient effect being not longer present in 9 weeks old calves. Because, however, particularly very young calves are prone to suffer from gastrointestinal disorders it is further worthwhile to investigate the importance of the described more diverse bacterial microbiome for the incidence and severity of diarrhoea at an early stage of the calves' life.

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22. Impact of a pectin supplementation *via* milk replacer on calves' performance and occurrence of diarrhoea

Einfluß eines Pektinzusatzes auf zootechnische Parameter und das Durchfallgeschehen von Kälbern

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Diarrhoea is a widespread problem in calves rearing causing a reduced growth performance. Pectins are supposed to have favourable effects on the intestinal health (1) by elevation of water binding, reduction of the passage rate, stabilization of a desired microbiom and increase of butyrate production within the intestine. Particularly highly methylated citrus pectin (HMCP) revealed favourable fermentation patterns with calves' faeces in vitro (2). The aim of this study was to investigate the impact of HMCP as a part of a milk replacer on the occurrence of diarrhoea and growth performance of calves.

Methods: 41 female Holstein-Friesian calves were randomly divided into control (CG, n = 18) and treatment group (TG, n = 23) at d4 of life. Until d14 of life, calves were individually housed in calf hutches, thereafter in groups (15/group, 4 lots - 2 KG, 2 VG). CG calves received a commercial milk replacer (MR; ingredients: 45% skim milk powder, 28.5% sweet whey powder, 17.2% vegetable fats, 4.9% partly de-sugared whey powder, 2.0% wheat flour, 0.5% wheat protein, 0.5% calcium carbonate, 1.4% premix; 200 g MR/L water) and TG calves an experimental MR with 1.2% HMCP in exchange for whey powder. The first eleven days, all calves could drink 3 times a day up to 3 L of liquid MR. From d15 until d63, the supply was slowly reduced to 2 L/d. Further, calves received a total mixed feed and hay *ad libitum*. They were weighed at d1, d14 and d63 of life. Consistency of faeces was recorded daily from d4 - d14 and faeces scored: (1) formed, pasty, (2) pasty thin, (3) creamy thin, (4) watery thin. A score > 2 are considered as diarrhoea. Milk intake, body weight (bw) and bw gain were analyzed by ANOVA (SPSS Version 20.0), start and length of diarrhoea and faeces consistency *via* Mann-Whitney test and Chi-square test.

Results: Between d15 - d63, TG *vs* CG calves consumed 0.7 L more liquid MR per day (6.1 ± 0.278 vs 5.4 ± 0.211 L/d; $P < 0.01$). Nevertheless, calves of both groups grew similarly throughout the trial (CG *vs* TG: 761 ± 0.2063 *vs* 766 ± 0.1601 g/d; $P > 0.05$). All calves except one per group suffered from diarrhoea, which started in CG and TG calves between d8 and d9 (8.1 ± 1.52 *vs* 8.8 ± 2.14 d of life; $P > 0.05$) and took slightly longer in CG calves (4.9 ± 1.34 *vs* 4.0 ± 1.94 d; $P > 0.05$). The faeces quality differed between TG and CG over the entire observation period (2.3 ± 0.565 vs 2.5 ± 0.634; $P < 0.05$) with particularly pronounced differences on d9 and d11 (d9: 2.4 ± 0.499 vs 2.9 ± 0.471; d11: 2.7 ± 0.449 vs 3.1 ± 0.676; $P < 0.05$).

Conclusions: Results suggest that HMCP as ingredient of a milk replacer for calves may enhance its intake with favourable effects regarding the alleviation of diarrhoea. We speculate that the voluntary intake of solid feed, however, was higher in calves consuming the more traditionally composed milk replacer. Otherwise, it would be impossible to explain why the growth performance was similar in both groups although the CG calves ingested lower quantities of milk replacer and suffered from diarrhoea for a longer time. Further, it needs to be investigated i) whether 1.2% HMCP enrichment of milk replacer indeed restricts the voluntary intake of solid feeds, and ii) if so, what that means particularly for the development of the rumen and thus the animals' future performance.

(1) BAUER E., WILLIAMS B.A., VOIGT C., MOSENTHIN R., VERSTEGEN M.W.A. (2010): Arch. Anim. Nutr.64, 394-411.
(2) Kieckhäven S., BÜSING K., ZEYNER A. (2013): Proc. Soc. Nutr. Physiol. 22, 101

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23. Glycaemic and insulinaemic response and feed intake patterns of sport ponies following iso-energetic intake of a low-starch mixed feed high in fat and fibre or oats

Glycaemische und insulinaemische Reaktion sowie Futteraufnahmeverhalten von Sportponys infolge isoenergetischer Aufnahme von stärkearmem aber fett- und faserreichem Mischfutter oder Hafer

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High glycaemic and insulinaemic blood levels are supposed to be critical for equines with reduced glucose tolerance and those prone to develop an equivalent endocrinopathy. Mixed feeds are requested which do not induce high postprandial (ppr.) glycaemic and insulinaemic responses, but may be suitable for additional energy and nutrient supply. Aim was to study effects of a low-starch mixed feed high in fat and fibre on feed intake patterns and ppr. glycaemic and insulinaemic responses compared to oats.

Methods: Six sport ponies (3 males, 3 geldings; age 6.3±3.0 years: body weight [bwt] 375±28.8 kg; body condition score 5.3±0.6 / 9) were individually housed in box stalls with wooden shavings as bedding and trained for 1 hour/d (supply of metabolizable energy [ME] 1.3-fold maintenance; maintenance: 0.52 MJ ME/ kg $bwt^{0.75}$, 1). Ponies were allocated per random onto 2 groups and received in 2 equal doses/d either oat grains (OG; semi-crushed; 1 g starch/kg bwt*meal; per kg dry matter [dm]: 398 g starch, 13.4 MJ ME/kg) or iso-energetic quantities of a low-starch mixed feed high in fat and fibre (FF; muesli; per kg dm: 48.6 g starch, 337 g acid detergent fibre, 95 g acid ether extract, 10.1 MJ ME/kg), according to a cross over design with period length of 3 weeks each. Hay covered the remaining energy need. At the end of each period, blood was sampled 1 hour after 500 g of hay was given and 30, 60, 90, 120, 180, 240, 300 min after the immediately subsequent ingestion of OG or FF meal. Blood plasma was stored (- 20°C) for later analysis of glucose (Hitachi 912) and insulin (Insulin-Coa-ACount-RIA-Kit). The area under the curve (AUC) was calculated until 120 and 300 min ppr.. Feed intake patterns were measured in 4 ponies per group by a modified halter (2). One-way ANOVA was performed.

Results: Ponies kept their bwt during the study ($P > 0.05$). OG was ingested faster than FF (min/kg dm: 8.8 ± 1.6 *vs* 15.9 ± 3.6; $P > 0.05$) with a tendency to be chewed at a higher frequency ($P > 0.05$). OG increased plasma glucose and insulin remarkably with peaks 90 and 120 min ppr. (5/6 ponies each), respectively, and complete return to baseline until 300 min ppr.. With FF there was rather a tendency for both variables to decline ppr.. AUC`s glucose and insulin until 120 and 300 min ppr. were, thus, higher with OG than FF ($P < 0.05$).

Conclusion: Results suggest that ponies may ingest a low-starch mixed feed rich in fat and fibre without any ppr. increase of plasma glucose and insulin. This might be favorable for equines with reduced glucose tolerance despite it needs to be validated in actually affected individuals. In the current study, ponies consumed the oat grains as fast as reported previously from horses, but not nearly as slowly as measured in ponies in the comparative study cited here (3). If this result would be confirmed by a further direct comparison of horses and ponies it would indicate that rapid consumption of starchy feed has yet been underrated as risk factor for ponies. The prolonged time for intake of the fat and fibre rich mixed feed is in any case advantageous. The physiologic importance of slightly declining or at least constant plasma glucose after ingestion of FF type feed particularly for exercised horses needs to be evaluated.

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24. Ensiling moist seeds of field beans and the influence of conservation on contents of oligosaccharides, tannins and vicine/convicine

Silierung feucht geernteter Ackerbohnenkörner und der Einfluss der Silierung auf den Gehalt an Oligosacchariden, Tanninen und Vicin/Convicin

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Ensiling of high moisture field bean seeds was examined as a low cost and environmentally friendly preservation method which additionally might be applied to reduce anti-nutritional factors.

Methods: Field bean seeds (variety 'Limbo') were harvested at approximately 65 % dry matter (DM). Proximate nutrients were analysed (in % DM: crude ash, 3.5; crude protein, 28.2; acid ether extract, 1.9; crude fiber, 8.6; starch, 41.9; sugar, 1.6). Cracked seeds (3 mm mesh size; 600 g) were ensiled on laboratory scale (1) in the following treatments: without additives (CON), with 2 % of molasses (MOL), inoculated with lactic acid bacteria (*Lb. plantarum*, DSM 8862, 8866, $3x10^5$ CFU/g; LAB) and a combination of both (MOL+LAB). Three bags per treatment were vacuum-sealed, stored at 20° C for 90 days and then used to prepare silage extracts (SE; 50 g of silage in 200 mL *aqua dest*. for 15 h at 5° C). Organic acids, ethanol and oligosaccharides (raffinose, stachyose, verbascose) were analysed by GC and HPLC (HPX-87C, Biorad, Hercules, USA) and pH values were measured within SE. Seeds and silages were photometrically analysed for tannins, phenols (2) and vicine/convicine (3). Analysis of variance with Duncan test for *post hoc* comparison of means was performed to assess the treatment effect (SPSS 14, Chicago, IL, USA). The level of significance was set at $P < 0.05$.

Results: Organoleptic evaluation of the silages showed good quality in all treatments tested. Despite high DM contents, pH values were reduced sufficiently (≤ 4.3). Concentrations of lactic acid (≥ 4.3 % of DM) in the control treatments were in accordance with the measured pH values. Butyric acid and propionic acid were not detectable and only low concentrations of acetic acid (≤ 0.4 % of DM) and ethanol (≤ 0.3 % of DM) were determined. Adding molasses did not affect silage quality, whereas inoculation with LAB led to a deeper pH decline and increased concentration of lactic acid ($P < 0.05$). As an overall effect, ensiling reduced concentrations of oligosaccharides, condensed tannins as well as total and tannin phenols ($P < 0.05$).

Conclusion: The results of the study suggest that field bean seeds can be successfully ensiled even with dry matter contents around 65 %. A particular advantage of this kind of feed conservation is that important anti-nutritional factors such as oligosaccharides and tannins are widely degraded. The use of LAB ensure a firm fermentation. Additional sugar sources are obviously not necessary to improve silage quality, probably because oligosaccharides are available for lactic acid fermentation.

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25. Effects of treating barley with citric or lactic acid on the resistant starch content and changes in nutrient composition

Effekte einer Behandlung von Gerste mit Zitronen- oder Milchsäure auf die chemische Zusammensetzung

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Question: There is an increasing interest to enrich foodstuffs with resistant starch (RS) due to its health-enhancing properties (1). Previous works showed that treating barley with lactic acid (LA) increased the content of RS but modified its chemical composition (2). In this work, we evaluated the effects of treating barley grain with LA or citric acid (CA) separately or together on starch characteristics and chemical composition on 4 different barley genotypes.

Methods: Quadruplicates of 4 different barley genotypes (50 g) were crushed, soaked in 60 ml of distilled water (control) or distilled water plus 5% (vol/vol) of LA, CA, or LA+CA (50:50) for 24 h. To determine effects of various acid concentrations in the steeping medium, quadruplicate samples of barley Calcule were additionally soaked in either 1 or 5% solutions (vol/vol) of LA, CA and CA + LA (50:50). Starch content was differentiated in RS and non RS (NRS) using the RS assay (Megazyme International Ireland Ltd. Co., Wicklow, Ireland). Chemical composition of all barley genotypes was determined. For the examination of the barley starch structure a scanning electron microscopy was used. Analysis of variance was carried out using SAS (9.2 version). Linear and quadratic orthogonal contrasts were tested using the contrast statement of SAS.

Results: Compared to the control (0.07%), the RS content of the genotype Susi increased after a LA treatment (0.5%; $P = 0.002$) whereas the RS content of the genotypes Espinosa (0.33 vs. 0.75%; $P = 0.008$) and Susi (0.82%; $P < 0.001$) increased after a CA treatment. The NDF content decreased after a treatment with CA at the genotypes Calcule (14.1% vs. 8.17%; $P < 0.001$) and Espinosa (11.97% vs. 9.67%; $P = 0.0161$) and after a treatment with LA at the genotypes Calcule (10.92%; $P < 0.001$) and Susi (11.64% vs. 8.32%; $P < 0.001$). With increasing LA concentration from 0% (control) to 1 and 5%, the amount of RS increased linearly, while treatments with CA did not show any effect. Compared to the control, ash, crude protein, and NDF contents decreased ($P < 0.05$) after acid treatments (contrast 1). The decrease in NDF was associated with an increase of soluble fiber fraction. RS and total starch ($P < 0.05$), but numerically also NRS ($P < 0.1$) were lower after a CA+LA treatment than after single corresponding treatments (contrast 2).

	Medium				SEM	p-values	
Nutrient (% of dry matter)	Control	CA	LA	CA+LA		Contrast 1	Contrast 2
Resistant starch	0.45	0.54	0.63	0.44	0.06	0.3807	0.0174
NRS	51.51	56.35	56.25	54.25	1.04	0.0230	0.0709
Total starch	51.96	56.88	56.87	54.69	1.01	0.0174	0.0476
Crude ash	2.27	2.12	2.03	2.08	0.02	<0.001	0.7824
Crude protein	11.41	10.33	10.17	10.59	0.09	<0.001	0.0005
NDF	14.10	8.17	10.92	12.35	0.31	<0.001	<0.001

Contrast 1: Control vs. all mediums; Contrast 2: CA and LA separate vs. CA+LA

Conclusions: Treatment with LA and CA increased RS and lowered ash and NDF contents of barley grain. Treatment with LA at a concentration of 5% showed the best ratio between RS and total starch amount, and deserves further investigations in vivo.

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26. Comparison of an indirect photometric method for determination of phytate with inositol phosphate determination in different corn conserves

Vergleich einer indirekten photometrischen Methode zur Messung von Phytat mit der Bestimmung von Inositolphosphaten in verschiedenen Maiskonserven

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The majority of P in corn occurs in the form of phytates, whereby storage procedures using fermentation techniques can lead to phytate-degradation (1). The predominant method used for phytate measurement is based on the decolarisation reaction between phytate and ferric ions. This method does not enable differentiation of phytic acid from partially dephosphorylated phytic acids such as $InsP_5$-$InsP_1$. Thus, the aim of the present study was to compare the phytate content of different corn conserves measured with an unspecific method with direct determination of inositol phosphates.

Methods: The corn conserves used were harvested from the same batch and prepared in the following way: freeze-dried directly after harvest ("fresh corn"), dried ("dried corn"), milled and ensiled ("corn silage") or tight-closed stored as whole grain ("TCS-corn"). The methods compared were: 1) The modified AOAC method by Latta and Eskin (2): Phytate was extracted from 5 g of corn samples by stirring in 100 ml 3.5% HCl for 3 h. After centrifugation at 6.000 rpm for 10 min, 1 ml of the supernatant was diluted 1:50 and 10 ml were run over an previously conditioned anion exchange column (Dowex 1X8 200-400 (Cl)). The column was washed with 15 mL 0.1 M NaCl. Subsequently phytate was washed out with 5 mL 0.7 M NaCl. 3 mL of this solution was mixed with 1 mL of Wade reagent and absorption was measured at 500 nm.

2) A modified method of Blaabjerg et al. (3) using HPIC for separation of $InsP_2$-$InsP_6$ and isomers of $InsP_3$-$InsP_5$: 0.5 g of each sample were extracted with 5 mL of 0.5 M HCl for 3 h and centrifuged at 5.000 rpm for 30 min using ultracentrifugation (Amicon® Ultra-2.0, Merck Millipore). Separation was performed on a HPIC CarboPac PA-1 analytical column. Inositol phosphates were eluted with a gradient of 5-100% CH_3SO_3H at a column temperature of 30 °C. The eluate was monitored using UV detection after post-column reaction with 0.1% $Fe(NO_3)_3 9H_2O$ in 2% $HClO_4$. The flow rates of the eluents and the post-column reaction solution were 0.5 and 0.33 mL min^{-1}, respectively. All samples were analyzed at least in duplicate with each method. Differences between phytate and $InsP_6$ contents were determined applying paired-t-Test.

Results: Phytate-P was on average 0.355 ± 0.043 g/kg higher compared to $InsP_6$-P (Table, p=0.001). Corn silage showed the lowest amount of phytate and $InsP_6$ (Table). Only small concentrations of lower phosphorylated inositol phosphates were determined. The predominant $InsP_5$-P was Ins(1,2,4,5,6) in all conserves, especially in corn silage and TCS-corn.

InsP-P	Fresh Corn	Dried Corn	Corn Silage	TCS-Corn	SEM
Phytate-P (g/kg)	2.63	2.77	1.50	2.40	0.1957
InsP6-P (g/kg)	2.24	2.37	1.13	2.14	0.1881

Conclusion: Ensiling of corn decreased phytate contents. The specific determination method showed lower concentrations compared to the widespread used photometrical phytate determination. The accumulation pattern of lower InsP-isomers may indicate that $InsP_6$ hydrolysis was related to 3-phytases.

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27. Effects of grinding method, particle size, and physical form of the diet on the distribution and relative frequency of intraepithelial lymphocytes in the small intestine of laying hens

Einfluss von Zerkleinerungsverfahren, Vermahlungsintensität und Konfektionierung des Futters auf die Verteilung und relative Häufigkeit intraepithelialer Lymphozyten im Dünndarm von Legehennen

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Question: New developments in the production and processing of feed for the use in the poultry sector are associated with changes in the structure of feed. To date limited information is available about the impact of feeding differently processed diets on the intestinal immune system of chickens. The aim of this study was primarily to characterize the different lymphocyte subsets in the small intestine of laying hens and to investigate the effect of feed processing on their distribution and relative frequency

Methods: Laying hens (Lohmann Brown) aged 20 weeks were fed 8 different experimental diets: coarsely ground mash, finely ground mash, coarsely ground and expanded feed, and finely ground and expanded feed, whereby each diet was produced using a hammer mill and a roller mill. After 21 days, hens (n=8 for each diet) were slaughtered and samples were taken from the duodenum and jejunum. On the basis of flow cytometric measurements, the distribution and relative frequency of different intraepithelial lymphocytes (IEL) were determined focusing on the characterization and differentiation of T cells, natural killer cells and natural killer T cells. Statistical analyses were conducted using SPSS (version 21.0, Chicago, IL). Data were analyzed by using a 3-factor ANOVA to control for interaction and single-factor effects ($2 \times 2 \times 2$ factorial arrangement). Differences were considered significant at $P < 0.05$.

Results: Regarding to the proportions of IELs in the duodenum and jejunum, the results show no differences among the feeding groups. The means of the different IEL populations were higher in the case of cells isolated from the jejunum.On average, a lower frequency of leukocytes were obtained from the duodenum compared to the jejunum (83 % vs. 88%) shown by the quantity of CD45+ cells. The percentage of cells expressing the CD3 receptor (T cells) ranged from 70 % in the duodenum to 75 % in the jejunum. Within the T cell population, αβ+ T cells and γδ+ T cells were detected, whereas higher percentages of γδ+T cells were found compared with TCRαβ1+ cells and TCRαβ2+ cells. NK cells, expressing the 28-4 glycoprotein, had percentages of about 7 % in both intestinal segments. There were also cells of about 5 %, which bore the CD3 receptor in addition with 28-4 glycoprotein (NKT cells). In the duodenum, 52 % of cells expressed the CD8α receptor and 56 % of CD8α+ cells were detected in the jejunum (cytotoxic T cells). T helper cells, identified by their CD4 receptor, were determined in proportions of about 8 % respectively.

Conclusion: The results of this study allow the characterization of different lymphocyte subsets in the small intestine of laying hens. The feeding of differently processed diets did not seem to influence the distribution and relative frequency of IELs in healthy hens. It might be conceivable that the effect of feed structure on the avian intestinal immune system will become more apparent in case of the occurrence of diseases.

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28. Replacement of soybean oil cake by *Hermetia illucens* meal in diets for layers

Ersatz von Sojakuchen mit Mehl von Hermetia illucens in einem Alleinfuttermittel für Legehennen

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Insect proteins are supposed to be valuable protein sources for monogastric animals (1). Among insects which could potentially be used for feeding livestock, the black soldier fly (*Hermetia illucens*) is particularly promising, because it can be reared on materials that are unsuitable for human nutrition (e.g. by-products from food processing) and because of its amino acid composition, which is similar to soybean (2). However, legal restrictions hinder the use of insect protein in compound feed for livestock at present.

Methods: A feeding experiment was carried out with experimental flocks of 10 laying hens at the end of their laying period. For each of four replicates, 30 white hens (Lohmann Selected Leghorn LSL; 64 - 74 weeks old) were purchased from commercial organic flocks and randomly distributed to one of three feeding groups. Each group was housed in an experimental unit equipped with perches, litter, nests, feeders and drinkers. Hens had permanent access to a covered outdoor area. Three types of experimental feed were produced: 'control': a standard control feed containing 36 g/100g soybean oil cake (dietary crude protein [CP] 200g/kg), 'H12', a feed containing 12g/100g Hermetia meal and 18 g/100g soybean oil cake (CP in diet: 200 g/kg), and 'H24', a feed with 24 g/100g Hermetia meal replacing 100% of the soybean (CP in diet: 230 g/kg). Hermetia meal was produced from air dried pre-pupae fed on by-products from pasta production; meal was partly de-fatted to contain 11 g/100g crude fat. The experiment started after one week of adaptation. Hens were then fed experimental diets for 3 weeks. Feed consumption was measured during the 3-week feeding phase. Egg production and animal condition were recorded daily. Live weight was recorded weekly. Data were analysed with the software SPSS© in a general linear model with group as fixed effect (n=4 replicates).

Results: A tendency for higher feed intake with H24 compared to control was found. Laying performance, feed intake per egg, egg weight and liveweight changes did not differ between the groups. No signs of health disorders occurred and mortality was zero.

Parameter	Group				Statistical values
	Control	H12	H24	S.E.	p-value
Feed intake [g/d]	107	116	131	9.8	0.091
Feed intake [g/egg]	134	148	159	16.7	0.327
Laying performance [%]	79.0	83.4	84.4	8.20	0.791
Egg weight [g]	65.9	67.2	68.7	1.78	0.303
Liveweight change [g/21 days]	-5.0	22.5	30.0	52.37	0.787
Mortality [%]	0	0	0	-	-

Conclusion: The partial or full replacement of soybean cake by meal from *Hermetia illucens* in a diet for layers did not affect their feed intake, feed efficiency nor laying performance and egg weights. These results indicate that insect larvae could serve as a valuable replacer for soybean products in layers diets. However, further research on long-term feeding effects and on resulting egg quality is necessary to approve insect larvae as a practicable source of feed protein.

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29. Influence of different dietary methionine to cystine ratios on growth performance, feed efficiency and protein deposition in meat type chicken

Einfluss verschiedener Methionin:Cystin-Verhältnisse im Futter auf das Wachstum, Futteraufwand und Proteinansatz von Masthähnchen

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

Studies assessing the ideal dietary methionine (Met) to cystine (Cys) ratio are scarce and recommendations vary between 48:52 and 57:43 [1]. NRC [2] recommended a Cys supply at 47 % of total sulfur containing amino acids (TSAA) for optimal feather formation of meat type chicken from 3 to 6 week of age. Due to suboptimal dietary Cys supply, Met will be degraded to form Cys. Consequently, the Met supply is inadequate for body protein synthesis and other metabolic functions, respectively. Therefore, the objective of the current study was to evaluate different dietary Met to Cys ratios on growth performance, feed efficiency and body protein deposition of fast growing meat type chicken.

Methods: The growth experiment was carried out with 210 male meat type chicken (Ross 308) over 35 days, divided in a starter (week 1-3) and grower period (week 4-5). The control diet (I) was limiting in Met supply (0.27/0.25% Met in the starter/grower diet) and based on corn, field peas, soybean meal and soybean protein concentrate. In total, three experimental diets with different supplements of Met and Cys (II: 0.075 % Met; III: 0.15 % Met and IV: 0.15 % Met + 0.16 % Cys) were under study. Growth parameters as well as feed intake were recorded weekly. At the end of the growth trial 105 birds were slaughtered for whole body analysis and calculation of body protein deposition. Results were statistically evaluated by one-way ANOVA (IBM SPSS Statistics 22) using post-hoc Tukey or Games-Howell test to identify significant differences ($p < 0.05$).

Results: The table summarizes the results of starter and complete experimental period, respectively. Generally, one-way ANOVA yielded $p < 0.0001$ for each of the parameters under study. As expected, chicken fed the Met limiting control diet (I) responded with lowest growth performance and feed efficiency data. Elevated Met concentrations (diets II; III) increased feed intake (FI) and body weight gain (BWG) significantly. Both feed conversion ratio (FCR) and body protein deposition (PD) were significantly improved by the raised dietary Cys ratio at the highest level of Met supply (diet IV).

Week	Diet	Met	Cys	Met:Cys	FI	Final BW	BWG	PD	FCR
		[g/kg diet]		[% of TSAA]	[g]	[g]	[g]	[g/d]	
1-3	I	2.8	3.0	48:52	39±4a	541±76a	23±4a		1.71±0.16a
	II	3.5	3.0	54:46	55±4b	901±68b	40±3b		1.37±0.04b
	III	4.2	3.0	59:41	60±3c	1013±51c	46±2c		1.30±0.03c
	IV	4.2	4.6	48:52	60±3c	1022±70c	47±4c		1.26±0.02d
1-5	I	2.7	2.9	48:52	63±7a	1057±198a	29±6a	4.86±0.95a	2.25±0.25a
	II	3.4	2.9	54:46	89±7b	1894±216b	53±6b	8.80±1.05b	1.71±0.11b
	III	4.1	2.9	59:41	102±3c	2403±89c	67±3c	11.45±0.41c	1.51±0.03c
	IV	4.1	4.5	48:52	101±4c	2499±85c	70±2c	12.26±0.43d	1.45±0.04d

Conclusion: Supplementation of Met to a Met limiting diet significantly improved growth performance parameters of fast growing meat type chicken independent on suboptimal Cys supply. However, an improved utilization of Met for growth, feed efficiency and body protein deposition was observed when the Cys supply was elevated. Generally, the results confirm observations about effects on dietary protein quality [3] and indicate that Cys supply above 50% of the TSAA could yield a sparing effect for Met in broiler diets.

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30. Amino acid composition of novel protein sources from thermal hydrolysis of pig and poultry by-products

Aminosäuregehalte neuer Proteinquellen aus der thermischen Hydrolyse von Schweine- und Geflügelnebenprodukten

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The worldwide growing demand of protein feeds in animal nutrition requires acceptance of animal by-products as a resource. Prohibitions of the European Communities (REGULATION (EC) No 999/2001) concerning animal proteins in animal nutrition, the direct use of these materials is not allowed. New technical processes based on thermal pressure hydrolysis provide new protein sources as protein hydrolysates. However, both thermal conditions of processing and composition of raw materials may provide varying amino acid (AA) content and AA efficiency. Corresponding to higher protein and fat yield at higher hydrolysis temperatures (HT) the risk of AA losses is abundant.

Methods: Raw materials (RM) under study were animal by-products (Category 3) of pork (AniPor; A) and poultry (AniPou; B) slaughtering which were hydrolysed for 2 hours at different processing conditions. Hydrolysis temperature was 130 °C and 160 °C, respectively (ANiMOX GmbH, Berlin). Resulting hydrolysates were centrifuged providing three phases (solid phase as sediment, liquid protein hydrolysate phase, phase of lipids). The liquid phase containing the hydrolysed protein was decanted and gently dried up to a dry matter (DM) content of about 50 %. Crude nutrients and AA were analysed according to the Regulation (EC) No 152/2009.

Results: The DM content of all products was about 52 %, crude ash content dependent on the raw material 3.65 % of DM (A) or 4.3 % of DM (B) and the nitrogen (N) content averaged at 16.7 % of DM. Using the common protein factor (6.25) for calculation of CP, yielded CP contents of >100 % of DM. Calculation of N in the protein sources from N content of the analysed individual AAs led to a mean N content of 15.2 % and corresponding to a more real CP content (94.8 % of DM). However, this phenomenon needs further attention for evaluating the protein sources. Analysed AA contents are summarized in the table. In contrast to other feedstuffs, both very high Gly content and relatively high concentration of Lys, Thr and Arg were observed. In contrast, Cys content was extremely low, indicating a Cys-degradation during hydrolysis. At higher temperature treatment the Cys content further declined, supporting assumed degradation. Surprisingly, the Trp content was elevated at 160 °C.

RM	HT	Amino acid concentration [% DM]														
	[°C]	Lys	Met	Cys	Thr	Trp	Arg	Ile	Leu	Val	His	Tyr	Phe	Asp	Gly	Pro
A	130	3.9	0.9	0.09	2.1	0.24	7.2	1.5	3.7	2.5	1.2	1.0	2.3	6.1	20.2	11.2
	160	3.9	0.8	0.06	2.1	0.41	6.4	1.7	4.3	2.9	1.3	1.4	2.6	5.1	17.9	10.4
B	130	3.8	1.1	0.12	2.3	0.19	7.0	1.6	3.5	2.1	1.0	0.9	2.2	5.9	19.6	10.7
	160	3.9	1.3	0.04	2.2	0.37	6.2	2.0	4.2	2.5	1.2	1.4	2.5	5.1	16.0	9.1
		Amino acid ratio in reference to Lys [%]														
A	130	100	23	2	55	6	185	37	95	65	32	27	60	157	517	287
	160	100	21	2	53	10	165	44	112	74	35	37	66	132	463	269
B	130	100	30	3	60	5	184	43	93	55	28	24	59	156	519	283
	160	100	33	1	56	10	160	52	108	64	31	36	65	131	414	234

Conclusion: Animal protein hydrolysates are expected to be an interesting protein source for animal nutrition. However, the thermal hydrolysis process needs optimization according both to a high protein yield and minimized damage on AAs. In consequence, AA data have to be discussed together with results of assessing the complex protein value [1, 2] of animal protein hydrolysates under study.

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31. Effect of dried mango peels on intestinal microbial metabolites in piglets

Wirkung von getrockneten Mangoschalen auf intestinale mikrobielle Metaboliten bei Ferkeln

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In animal nutrition, using by-products from the food industry is common practice. With a worldwide production of 42 million tons in 2012, mango fruit is one of the major tropical fruits being mainly produced in Asia, Africa and America (1). Mango peel has been suggested as a good source of dietary fiber (2). The mango dietary fiber contains high amounts of total extractable polyphenols (70 g/kg), ß-carotene (~ 50 mg/kg), soluble dietary fiber (281 g/kg), and has a high water-holding capacity (11.4 g/g dry matter) (2,3). To assess the effects of dried mango peels supplemented to animal nutrition, an *in vitro* and *in vivo* study were conducted.

Methods: The *in vitro* experiment was based on short-term incubations under anaerobic conditions. Hungate tubes were filled with buffer solution, diluted with fecal inoculum from pigs (final dilution 1:100) and diluted test material (final concentration 0.2 %). After 24 h, the incubation was stopped, the pH-value was measured, and samples for the determination of the bacterial metabolites were taken. For the *in vivo* trial, ten weaned piglets were used in each group. The control group without supplement was compared to group B, where 2 % of mango peels were added. After a feeding period of four weeks, the piglets were sacrificed, and digesta samples were taken from the gastrointestinal tract. The contents of short chain fatty acids were quantitated by gas chromatographic analysis (GC Model 6890N, Agilent Technologies, Santa Clara, CA, USA). Means were compared by the Kruskal-Wallis test using SPSS software (version 21.0, Chicago, IL, USA).

Results: In the *in vitro* study, supplementation of mango peel resulted in increased amounts of short chain fatty acids compared to the control, where only fecal inoculum was incubated. The pH value in the incubations containing mango peel decreased ($p<0.001$) to 6.52 (6.42 - 6.67) compared to control (6.82 (6.78 - 6.95)).

Table 1: Selected metabolite concentrations after ***in vitro*** incubation of fecal extracts with mango peel (n = 5)

	Parameter [mmol/L]	Control	Mango peel	p value
	Acetic acid	4.01 (0.86 - 7.86)	6.36 (2.51 - 7.56)	0.075
	Propionic acid	0.41 (0.17 - 0.79)^{a}	1.07 (0.30 - 1.81)^{b}	0.002
	n-Butyric acid	0.12 (0.09 - 0.17)^{a}	0.42 (0.17 - 0.93)^{b}	0.000
	SCFA (total)	5.23 (1.83 - 9.53)^{a}	8.46 (4.44 - 14.2)^{b}	0.008

^{a,b} = Median values with different letters indicate significant differences among both groups ($p<0.05$)

Due to high individual variation in the *in vivo* trial, statistical differences for bacterial metabolites were insignificant.

Table 2: Selected metabolite concentrations in the intestine of piglet fed a diet containing 2 % mango peel (n = 5)

	Parameter [µmol/g]	Control	Mango peel	p value
Jejunum	Acetic acid	0.96 (0.52 - 23.0)	3.25 (0.79 - 9.07)	0.602
	Propionic acid	0.09 (0.00 - 0.16)	0.05 (0.03 - 0.10)	0.753
	n-Butyric acid	0.00 (0.00 - 3.44)	0.00 (0.00 - 0.65)	0.814
	SCFA (total)	1.17 (0.79 - 26.6)	3.46 (1.04 - 9.92)	0.602
Caecum	Acetic acid	60.7 (54.8 - 65.5)	62.0 (60.6 - 69.3)	0.175
	Propionic acid	33.1 (30.6 - 36.0)	35.5 (26.9 - 37.3)	0.917
	n-Butyric acid	14.1 (10.8 - 14.4)	14.6 (8.55 - 16.1)	0.465
	SCFA (total)	113 (100 - 114)	115 (97.3 - 126)	0.347

Conclusion: Fermentability of mango peel by the intestinal microbiota has been demonstrated by the present *in vitro* study. Consequently, mango peels proved to be a good source of dietary fiber. However, the effect on bacterial fermentation profiles could not be statistically confirmed by the *in vivo* study, due to the individual variation and the high standard deviation of the measured parameters. Therefore, further investigations are required to evaluate putative effects of mango peels on intestinal microbial metabolites in piglets.

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32. Effect of age at experimental induction of exocrine pancreatic insufficiency in young pigs on growth, body composition (relative weight of the gastrointestinal tract) and serum leptin concentration

Einfluss des Alters bei experimenteller Auslösung der exokrinen Pankreasinsuffizienz bei jungen Schweinen auf das Wachstum, die Körperzusammensetzung (relativer Anteil des Magen-Darm-Traktes) und die Leptinkonzentration im Serum

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In former studies (Mößeler et al. 2012, 2013) an elongation of the small intestine and an increase of relative weight of the gastrointestinal tract (GIT) was found in piglets with experimentally induced pancreatic exocrine insufficiency (PEI). The aim of this study was to compare different parameters of growth and relative weight of GIT (either with or without digesta) in control pigs and pancreatic duct ligated pigs (surgery either at age of 7 weeks or 16 weeks). As Fedkiv et al. (2009) stated that growth of older pigs is not affected by PEI, special emphasis was given to the group of pigs that underwent surgery at the age of 16 weeks. Leptin in serum was measured to check, whether changes due to PEI affect endocrine status.

Methods: Overall 26 piglets were used in this study. In 9 pigs the pancreatic duct was ligated (PL) at the age of 7 weeks (PL 7), while 8 pigs underwent surgery at an age of 16 weeks (PL 16). In controls a sham OP was performed at 7 weeks of age. None of the PL-pigs received pancreatic enzyme replacement therapy. A complete diet (% of dry matter: 37.3 starch, 11.5 crude fat and 20.3 crude protein) was fed (pair feeding; last two weeks: ad libitum feeding). Animals were euthanized at the age of 26 weeks and dissected. Body measures were determined using a tape; leptin concentration was measured in blood samples taken at dissection by use of a porcine specific Leptine ELISA (Sea084Po, cloud-clone corp., Houston, USA).

Results: PEI significantly reduced growth (body weight [bw] and body length) with an effect of age of the pigs at surgery (more distinct effects in PL 7). GIT (including digesta) accounted for about 8 % of bw in controls while it was more than doubled in PL 7-pigs. The relative tissue mass of GIT (emptied GIT) was also higher in PL compared to controls. Leptin concentration in serum was significantly lower in PL-pigs with no sign. effect of duration of PEI or body weight (see table 1).

Table 1: Different parameters of growth, relative weight of the GIT (with or without digesta) and serum leptin concentration of control pigs and pancreatic duct ligated pigs at the age of 26 weeks

	Control (n=9)	PL 7 (n=9)	PL 16 (n=8)
Body weight (kg)	117 ± 8.07a	49.5 ± 22.2c	96.4 ± 9.89b
Length (nose to tail; m)	1.43 ± 0.06a	1.15 ± 0.13c	1.38 ± 0.06b
Breast perimeter (cm)	108 ± 2.46a	76.9 ± 12.4c	99.5 ± 5.10b
Relative weight of filled GIT (% of bw)	7.95 ± 0.86a	17.3 ± 2.00c	14.4 ± 2.75b
Relative weight of emptied GIT (% of bw)	3.46 ± 0.41a	7.80 ± 1.30c	5.67 ± 1.97b
Leptin concentration (ng/mL)	12.7 ± 7.03a	0.72 ± 0.76b	1.21 ± 1.54b

Different letters mark significant effects of treatment (p

Different parameters of growth were markedly altered by untreated PEI. In PL-pigs that underwent surgery early in life the body measures were shortest and relative weight of GIT was highest. Nonetheless growth of PL 16 pigs was also impaired compared to controls. Interestingly leptin concentrations did not differ between the groups of PL-pigs although bw differed markedly.

Conclusion: The massive increase of the mass of GIT in case of PEI should be taken into account whenever growth or bw development is measured in any case of impaired digestion as it might result in a massive overestimation of bw gain. The different parameters of growth indicate that PEI also impairs growth of older pigs (16 weeks of age at surgery) but largest effects were found in PL 7. Leptin levels show a distinct effect on endocrinologic status in PL 16 pigs - indicating additionally that bw alone is not suitable to estimate nutritional status.

References: Available at request

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33. Correlations between performance parameters and level of faecal Lawsonia intracellularis shedding in clinically-apparent, clinically-inapparent and vaccinated piglets

Korrelationen zwischen Leistungsparametern und Höhe der fäkalen Lawsonia intracellularis-Ausscheidung in klinisch auffälligen bzw. unauffälligen und geimpften Ferkeln

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Piglets with a clinically obvious *Lawsonia intracellularis (L.i.)* infection excrete high counts of this pathogen with faeces. Increasing numbers of *L.i.* in pig faeces, quantified with qPCR, showed a strong negative correlation with average daily weight gains (1). This study tested the hypothesis that the known correlations between performance and qPCR results are also valid for clinically inapparent and vaccinated animals.

Methods: In 3 consecutive trials a total of 27 potentially naturally *L.i.* infected pigs (body weight: 19.0 ± 1.50 kg; 9 pigs/group) were fed a conventional diet ad libitum (XP: 176 g, XF: 23.5 g, XL: 33.6 g, ME: 13.8 MJ/kg diet). Animals were allotted to 1 of 3 groups: not vaccinated, without clinical findings = $VAC^{-}CF^{-}$; with clinical findings (soft to liquid faeces) = $VAC^{-}CF^{+}$; vaccinated (Enterisol^{Ó}Ileitis; as suckling piglet) = VAC^{+}. Pigs were housed individually and fed the diet for 10 d (5 d adaptation/5 d faeces collection) to determine performance parameters and digestibility of nutrients (2). The *L.i.* excretion was analysed in an aliquot of faeces from the collection period by qPCR. Statistical analyses were performed by one-way ANOVA (procedure GLM; significant for p £ 0.05). Correlations are given by means of the Pearson (normal distributed) or the Spearman correlation coefficient (not normally distributed).

Results: Faecal shedding of *L.i.* was found in all groups (25 of 27 animals) with the highest numbers of genome equivalents seen in group $VAC^{-}CF^{+}$ (lg genome equivalents: 7.70±1.65). Animals in group $VAC^{-}CF^{+}$ had the significantly lowest dry mater content (DM) in the faeces (211±19.8 g/kg) and showed the lowest average daily weight gains (785±137 g).

Table 1: Correlations between numbers of genome equivalents and DM of faeces or rather average daily weight gains in pigs shedding *L.i.*

	$VAC^{-}CF^{-}$		$VAC^{-}CF^{+}$		VAC^{+}	
lg genome equivalents L.i. (in 1 g faeces)	5.83	± 2.35	7.70	± 1.65	6.00	± 2.89
DM content of faeces (g/kg)	245	± 16.8^{a}	211	± 19.8^{b}	236	± 18.4^{a}
Pearson corr. coeff. „r“ lg genome equivalents vs. DM		-0.15		-0.56		0.17
feed intake (g/d)[1]	1.21	± 0.12	1.16	± 0.15	1.30	± 0.12
Spearman corr. coeff. „r“ lg genome equivalents vs. feed intake		0.06		-0.35		0.49
average daily weight gains (g)	857	± 86.3^{ab}	785	± 137^{b}	894	± 73.4^{a}
Pearson corr. coeff. “r” lg genome equivalents vs. average daily weight gains		0.01		-0.58		0.43

[1] due to technical problems, only trial 2 and 3 were considered

In animals with a clinically apparent infection, there were moderate (feed intake) or clear linear negative correlations for the analysed performance parameters, whereas in non-vaccinated pigs with no clinical signs of infection only poor or rather no correlations could be seen. In vaccinated animals there was a moderate linear positive correlation between feed intake and amount of excretion (r=0.49) and between ADWG and faecal *L.i.* shedding (r=0.43).

Conclusion: In this study there was a negative correlation between performance and *L.i.* counts in faeces in non-vaccinated, clinically apparent animals, confirming the results of a published work (1). In vaccinated, clinically healthy animals the same correlation was missing.

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34. Digestibility of diets containing different proportions of differently processed whole-plant corn silage in growing pigs between 40 and 60 kg bodyweight

Nährstoffverdaulichkeit von Rationen mit unterschiedlichem Anteil von unterschiedlich bearbeiteten Mais-Ganzpflanzensilagen bei jungen Schweinen

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Whole-plant corn silage (WPCS) is a common basis of rations for ruminants and is predominantly used in dairy cattle and fattening bulls. WPCS is an affordable fiber source for pigs rich in starch that can be used to elevate the crude fiber supply. Progress in the milling techniques such as wet-grinding after soaking and the fact, that compatible liquid-feeding systems are widely used in fattening pigs and sows, favor its use for pigs. Higher amounts of fiber in a ration might contribute to animal welfare, reduce abnormal and aggressive behavior in pigs and improve the gut health of animals (1). Exact values for the apparent digestibility (aD) of nutrients of WPCS in growing pigs are scarce in the recent and old literature.

Methods: 4 barrows (BW 43.6 ± 4.5 kg, age 99.8 ± 6.70 days) were housed in individual pens and fed restrictively(ca.1.4 of maintenance requirement of energy)a botanically and chemically identical concentrate (903 g DM/kg FM, per kg DM: 42.8 g CF), which was used as the basis in the first aD trial. The results were used to calculate the aD of the WPCS. The different diets were fed successively, it means at different ages. After a 5- up to 10-day period of adaptation to the diet, the total collection of faeces was performed the following 5 days. Two batches of WPCS were tested: WPCS 1 (392 g DM/kg FM; per kg DM: 171 g CF, 378 g starch), finely ground in a CCM grinder before ensiling;WPCS 2a (66.4 g DM/kg FM; per kg DM: 183 g CF,362 g starch), soaked and wet-ground as silage; WPCS 2b (306 g DM/kg FM, per kg DM: 205 g CF,363 g starch), conventionally chopped WPCS. The particle size of different WPCS was determined by wet sieve analysis. The aD of organic matter, crude protein, crude fiber, NDF and ADF was determined. Statistical analysis was performed with ANOVA-procedure in SAS 9.3 for Windows. Only the aD of the last three trials was statistically analysed, due to the fact, that the barrows had a comparable bodyweight (>50 kg), therefore a similar development of the gastrointestinal tract, and the same amount of DM (45%) of the ration was substituted with WPCS.

		Barrows (n=4)		Apparent digestibility of organic matter (%)	
% of DM	WPCS	BW (kg)	age (days)	Whole ration	WPCS
0	-	43.6 ± 3.9	99.8 ± 6.7	88.6 ± 2.3	-
15	1	48.8 ± 5.0	122 ± 6.7	82.8 ± 1.8	51.7 ± 3.1
33	1	42.8 ± 3.6	110 ± 6.7	77.3 ± 2.4	56.3 ± 3.5
45	1	53.1 ± 4.4	132 ± 6.7	73.8^{a} ± 1.3	57.4^{a} ± 0.3
45	2a	56.9 ± 5.0	147 ± 6.7	75.0^{a} ± 2.3	58.1^{a} ± 3.6
45	2b	60.9 ± 6.1	161 ± 6.7	75.7^{a} ± 2.5	59.9^{a} ± 4.6

1 = WPCS finely-ground, 2a = WPCS wet-ground, 2b = WPCS chopped, ± = SD
^{a,b} indicate significant differences ($p<0.05$)

Results: The wet sieve analysis showed that the percentage of fine particles (< 0.2 mm) was highest in WPCS 2a(1: 37.5% / 2a: 46.0% / 2b: 30.5%). The higher the dietary level of WPCS in the ration the more the aD of OM, CP, CF, NDF and ADF of the whole ration was reduced. The calculated values of aD of the WPCS showed a raising trend with higher substitution. The NDF digestibility of the WPCS was highest (38.1%) for pigs weighing ca. 60.9 kg.

Conclusion: This study showed that WPCS is a valuable feed stuff for growing pigs, if a higher fiber supply is intended. However the interpretation of the results is difficult because of the different age/bodyweight at which the aD was tested. The technique of diminution of the WPCS from batch two had no significant effect on the aD of the organic matter. The aD of OM of WPCS as found earlier (62%; 2) is comparable to the values determined here. The aD of OM of the whole ration and the WPCS of all trials mostly correspond with the expected values from the regression equation which considers the crude fiber content (3). Despite the fact that WPCS 2b has a higher crude fiber content than 2a the aD of OM was similar. The calculated ME for WPCS 2a is 9.81 MJ/kg DM for 2b 10.35 MJ/kg DM. In the future new techniques like the modification of WPCS with a pulsed electric field might raise the efficiency of feeding WPCS to pigs. Up to now WPCS was not offered exclusively, but that is the focus of upcoming experiments.

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35. Effects of 2-stage grinding in production of compound feeds on digestibility, performance and gastric health in weaned piglets

Einfluss einer 2-stufigen Vermahlung in der Mischfutterproduktion auf die Verdaulichkeit, Leistung und Magen¬gesundheit bei Absetzferkeln

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Introduction: Grinding of raw materials in production of compound feeds (COF) for pigs has a significant impact on physical properties of the diet as well as on animal health and performance. In the past the use of hammer mills for grinding diverse ingredients in swine feeding was recommended due to technological and logistical advantages, but also to achieve a high digestibility of the diet (Healy et al., 1994). Undesired consequences of finely ground diets are also well known in terms of stomach health (Wondra et al., 1995). Coarsely ground diets in pig feeding are increasingly discussed because of favourable effects regarding stomach health and reduction of prevalence of *Salmonella spp*. in pig stocks. The wedge-shaped disc mill consists of two horizontal cylinders that rotate oppositely to each other with various speeds. A major advantage is the low operating energy (< 3 kWh/t; Hoffmann et al., 2011). But like specified for roller mills, also wedge-shaped discs mills reduce ductile components incompletely. In this trial the use of a hammer mill in a second step should diminish the proportion of coarse particles and husks. The aim of this study was to evaluate potential effects of a 2-stage produced COF, in comparison to a conventional one, on digestibility, performance and gastric health in pigs.

Methods: 16 weaned barrows at the age of 40 days (BW: 8.90 ± 1.00 kg) were housed individually and offered a botanically and chemically identical diet (ME: 15.6 MJ/kg, 21 % CP in DM), which differed in grinding-type, -intensity and further compaction, over a period of 4 weeks (total duration of trial). COF_H was produced like a conventional finely ground compound feed by hammer mill and fed to controls (n=5). Ingredients of COF_{WH} for the second group of piglets (n=5) were ground using a wedge-shaped disk mill (first step) and after that only sieve separated coarse particles were ground by hammer mill (second step). Both diets were offered as dry mash. For the third group of piglets (n=6) COF_{WH} was pelleted (3 x 40 mm die), henceforth called COF_P. The apparent digestibility was determined by collecting the whole faeces of five days. The stomach mucosa of the pars nonglandularis (PN) was evaluated by a macroscopic scoring system according to Grosse Liesner (2008), modified by Wintermann (2011). Statistical analyses were done using the NPAR1WAY-procedure of the SAS software, resp.

Results: The particle size distribution (PSD) of the diets, performance data [dry matter (DM) intake, daily weight gain (DWG)] and apparent digestibility (total GIT) are presented in the following table:

Group	PSD[1] feed, (%)			DM intake[2] (kg)	DWG[2] (g)	App. digestibility[3] (%)			FCR	PSD[1] faeces, (%)		
	> 1 mm	0.20 -1 mm	< 0.2 mm			OM	CP	starch		> 1 mm	0.20 - 1 mm	< 0.2 mm
COF_H, n=5	31.1	32.5	36.4	20.1[a] (± 1.8)	498[a] (± 69.1)	84.8[a] (± 3.3)	79.9[a] (± 1.8)	98.9[a] (± 0.2)	1.65[a] (± 0.05)	19.7[a] (± 1.2)	23.8[a] (± 1.7)	56.5[a] (± 1.5)
COF_{WH}, n=5	48.3	21.7	29.9	22.3[a] (± 2.0)	587[a] (± 61.5)	86.9[a] (± 1.5)	80.6[a] (± 4.2)	98.7[a] (± 0.4)	1.57[a] (± 0.09)	21.8[a] (± 4.7)	18.5[b] (± 1.4)	59.7[a] (± 4.4)
COF_P, n=6	46.5	25.6	27.9	23.7[a] (± 2.2)	514[a] (± 86.7)	85.8[a] (± 1.2)	77.9[a] (± 2.3)	98.8[a] (± 0.2)	1.58[a] (± 0.07)	9.6[b] (± 1.9)	27.0[c] (± 0.6)	63.4[b] (± 1.5)

[a, b, c] indicate significant differences ($p < 0.05$);[1] measured by wet screening analysis; [2]over 4 weeks, [3]collecting 5 days

Regarding DM intake, apparent digestibility, DWG and FCR there were no significant differences between all three groups, although group COF_{WH} showed the highest DM intake, DWG and also digestibility rates. The PSD of faeces showed a significant difference in group COF_P. The proportion of particles > 1 mm was appreciably lower in contrast to group COF_{WH} and COF_H. Furthermore in COF_{WH} absolutely no signs of mucosal alterations in the region of pars nonglandularis were detected (score: 0).

Discussion and conclusion: The digestibility was not negatively influenced by the new 2-stage grinding by wedge-shaped disc- and hammer mill. Although the proportion of coarse particles (> 1 mm) was almost equal in the diets of groups fed COF_{WH} and COF_P, in faeces of piglets fed pellets the proportion of coarse particles was significantly lower. The reasons are not clear up to now but potential effects of thermal treatment while pelleting (gelatinisation, viscosity) can be discussed. But the low proportion of coarse particles in faeces of group COF_P was not related to higher digestibility. In comparison to conventional hammer mill diminution, 2-stage grinding by wedge-shaped disc mill reduces energy costs about 30 % and had no negative effects on FI, digestibility and performance but key advantages on gastric health.

Grosse Liesner (2008) - Healy et al. (1994) - Hoffmann et al. (2011) - Wintermann (2011) - Wondra et al. (1995)

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36. The presence and localization of type 1 cannabinoid receptor in the mandibular gland of young pigs: influences of different physical form of diet

Vorkommen und Lokalisierung von Typ-1- Cannabinoid-Rezeptoren in der Speicheldrüse junger Schweine unter dem Einfluss einer unterschiedlichen Mischfutterstruktur

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Introduction: The endocannabinoid system is a complex system consisting of endogenous molecules named endo-cannabinoids (where the main representative is anandamide) and cannabinoid receptors. The latter in particular belong to the family of the G protein-coupled receptors and are membrane receptors; they are the type 1 receptor or CB1 and the type 2 receptor or CB2. These receptors are also activated by exogenous molecules, mainly of plant origin, commonly referred to as eso-cannabinoids. The CB1 is expressed mainly in the brain but also in some peripheral organs: the lungs, liver and kidneys, endocrine and salivary glands, spleen, heart, reproductive and gastrointestinal system. The CB2 is expressed mainly in the immune system and in hematopoietic cells. In particular, the localization of CB1 in the salivary glands with involvement of the epithelial cells of the ducts was explained as the cause of the diminishing effect of endocannabinoids in the salivary secretion, as observed in men who use cannabis. The aim of the present study was to test whether different physical forms of one diet might influence the expression of CB1 in the mandibular glands as the expression of a different stimulation of the secretory activity of the mandibular gland in growing pigs.

Methods: The experiment was conducted using 32 growing pigs (initial BW: 8.30±0.83 kg) fed ad libitum for 4 weeks with one of the four experimental diets. The four diets were identical for chemical and botanical composition but differed in the physical form: 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. The daily intake of food was higher in the "CM group" even if not significantly. At the end of the experimental period, the animals were slaughtered, 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 serial sections, utilising the primary goat polyclonal antibody anti-CB1 the avidin-biotin-complex and the DAB as the chromogen.
Sections in which the primary antibody was omitted were used as controls of unspecific staining.

Results: The immunohistochemical study showed a strong positivity for CB1 in the mandibular glands of the animals examined. In particular a difference was evident in the localization of the CB1 immuno-positivity among the animals fed with the coarser diet (CM diet) and the animals fed with diets more finely ground (FP, CP, CE diets). In the animals fed with CM diet the CB1 immuno-positivity was localized in the serous cells, while the mucous cells and the ductal epithelium appeared to be negative. In all other animals, in particular in those fed with the FP and CE diet, CB1 immuno-positivity was no longer present in the glandular adenomere but in the ductal epithelium located particularly in the cytoplasm of the epithelial cells near or on the apical cell membrane. Immuno-positivity for CB1 was not observed in the connective tissue or in the sections utilized as negative controls.

Conclusions: The CB1 expression seems to be influenced by the physical characteristics of the diets with a positivity that appears to be differently localized in the glands and there is an increased expression in the ducts in the animals fed with FP and CE diets. This preliminary result leads us to speculate that CB1 is involved in the control of pig salivary secretion, via endocannabinoids, and that these molecules most likely represent an important link between the physical state of the diet and salivation, as shown by the different expression and localization of CB1 in the four groups of animals.

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37. Influence of starch source on the *in vitro* production of skatole and indole by porcine fecal bacteria

In vitro-Untersuchungen zur Bedeutung der Stärkequelle für die Produktion von Skatol und Indol durch die fäkale Mikroflora von Schweinen

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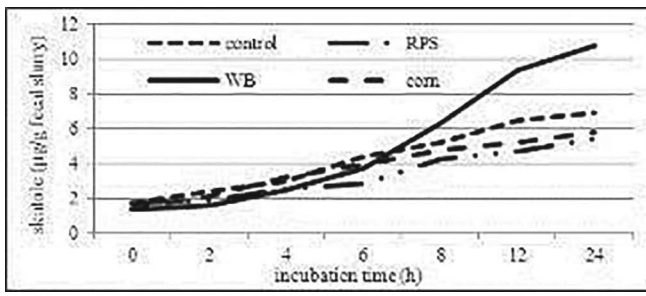
Question: In regard to the amendment of the animal welfare law and the ban of the castration without anesthesia from 2019 on, the fattening of boars is still an option. But up to now, the risk of the typical "boar taint", deriving mainly from skatole and androstenone, is still not solved. One possibility to reduce skatole production in the hindgut and therefore skatole levels in the carcass is the inclusion of raw potato starch or inulin in the diet [1]. The aim of this study was to investigate potential effects of further starch sources on the production of skatole in an *in vitro* approach since it is known that higher starch amounts in the hindgut can also be archived by coarse grinding of the grain.

Methods: In 3 consecutive fermentation trials fresh feces (400 g total weight) from pigs fed a standard soy bean-cereal based diet were blended in a CO_2-atmosphere with a mineral salt medium according to [2] to give a 10 % (wt/vol) fecal slurry. The homogenate was filtered to remove crude particulate material. Glass bottles (2 L working volume) from the DAISY^{II} Incubator (Ankom Technology) were flushed with CO_2, filled with 1 L fecal slurry and 51 mg L-tryptophan/L were added [3]. To 3 bottles the equivalent to 10 g starch in form of raw potato starch (RPS), a 1:1 mixture of wheat and barley (WB) or corn was added to the fecal slurry. The 4th bottle served as control. Bottles were incubated for 24 h by 38 °C. All following manipulations were done under a constant flow of CO_2. Samples were taken after 0, 2, 4, 6, 8, 12 and 24 h of incubation to determine concentrations of skatole, indole and short chain fatty acids. Statistical analysis (ANOVA, Proc GLM) was performed with SAS 9.3 for Windows and differences stated significant when $p < 0.05$.

Results: The addition of WB to the fecal slurry resulted in the lowest pH values and the highest concentrations of SCFAs throughout the incubation period; starting at 4 h, differences were always significant between WB and control and mostly significant compared to RPS. Differences in the skatole production became obvious after 8 h of incubation (Figure 1). In total, in this group the highest amount of tryptophan was metabolized to skatole over 24 h (mg/kg fecal slurry; WB: 14.7±4.71; control: 8.02±3.46; corn: 6.74±1.11; RPS: 5.68±0.873). Regarding indole, concentrations were lowest in WB and already differed, at least numerically, after 4 h. Interestingly, indole concentrations began to drop in all groups after 6 to 8 h of incubation. Week, but significant correlations between skatole and butyric acid (r=0.3, p=0.022) as well as skatole and propionic acid (r=0.41, p=0.001) existed. In contrast, negative correlations between indole and all SCFAs were highly significant ($p < 0.0001$; r-values between -0.574 and -0.750).



Conclusion: The starch source had major impact on the *in vitro* fermentation pattern, and in this case of special interest, on the production of skatole and indole. For this approach it should be kept in mind that the fecal bacteria was not adapted to all starch sources used and perhaps therefore not able to ferment the different starches in comparable extend. Nevertheless it can be concludes for the *in vivo* situation that - besides further mechanisms influencing the production of skatole in the hindgut - not only the amount, but also the kind of starch in the digesta seems to be of particular interest.

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38. Influence of supplementing diets with mineral phosphorus and phytase on gastrointestinal tract microbiota of broilers

Auswirkungen von mineralischem Phosphor und Phytase im Futter von Broilern auf die gastrointestinale Mikrobiota

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Chicken gastrointestinal environment harbours unexplored and complex bacterial communities. The interactions between the species, that include human and animal pathogens, are of great importance in regards to animal welfare and for safety reasons. They play an important role in immune responses, gut morphology, nutrition and chicken growth and performance [1]. The use of phytase as poultry feed additive is common. Commercial poultry production normally uses diets supplemented with 500 to 750 phytase units (FTU) per kg and dosages largely exceeding this standard are only rarely reported. The addition of mineral phosphorus (P) and phytase can make an impact on gastrointestinal tract ecology and previous studies have shown microbial activity modulation [2]. This study aimed to characterize the differences in crop, jejunum, ileum and ceca microbial communities between broiler chicken fed diets supplemented with phytase and a mineral P source.

Methods: Dietary treatments were based on two maize-soybean meal basal diets; one containing P only from plant sources (BD-) and the other supplemented with P from monocalcium phosphate (MCP) (BD+). Treatments consisted of the BD- and BD+ supplemented with 0, 500 or 12,500 FTU of an *E. coli* phytase/kg of feed. Unsexed broiler hatchlings were allocated to 48 floor pens in groups of 20. Until day 14 birds were fed with a commercial diet and at day 15 the pens were randomly assigned to the 6 dietary treatments. Digesta samples for microbial profiling (crop, jejunum, ileum and ceca) were taken at day 25 after birds being euthanized by carbon dioxide asphyxiation and the digestive tract dissected. Samples of each segment from 4 chicken/treatment were pooled and stored at -80°C. Total nucleic acids were extracted using a commercial kit. Samples were subjected to amplicon pyrosequencing using Roche GS FLX++ technology. Phylogenetic analysis of the 16S rRNA gene sequences was assessed using Mothur [3].

Results: Phytase addition favoured the abundance of microorganisms belonging to *Aeromonadaceae* and *Flavobacteriaceae* in the crop, however diminished the abundance of lactobacilli. There was no dietary treatment effect in the jejunum bacterial community, being this niche colonized mainly with *Lactobacillaceae* (>99%). A shift in the ileum microbial community was observed with BD- diets, where the abundance of microorganisms belonging to *Enterobacteriaceae* and *Peptostreptococcaceae* was increasing with phytase addition. The most significant difference in community structure was observed in the ceca, mainly colonized with *Bacteroidaceae*, *Ruminococaceae* and uncultured *Clostridiales* (circa 80% of total community). The abundance of *Erysipelotrichaceae* was decreasing with phytase addition in BD- and BD+ diets and phylotypes belonging to *Bacteroidaceae* were more abundant once phytase was added to the diet. Exploring the global bacterial community structure of the 24 samples using ordination revealed the presence of 3 distinct clusters comprising crop, ceca and jejunum/ileum sections. Analysis of similarity confirmed a significant difference between each section (R=0.756, p=0.001). The phylotypes contributing significantly to the observed differences were *Lactobacillus crispatus*, *L. salivarius*, *L. taiwanensis*, *Bacteroides fragilis*, *L. aviaries* and *Shigella flexneri* that were detected with different abundances in each section.

Conclusions: The microbial community was influenced by the addition of phytase and MCP. A distinct microbial assemblage on each section was observed. There was an indication that phytase supplementation, but not MCP, affected microbiota in the crop and caeca. Phytase and MCP had only minor effects in the ileum.

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39. Comparison of different feed efficiency traits in broiler chickens

Vergleich verschiedener Futtereffizienz-Klassifizierungen beim Masthuhn

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Improving feed efficiency is of great interest due to increasing feed costs and the necessity to reduce the environmental burden of animal production. Feed conversion ratio (FCR) is commonly used to measure feed efficiency in broiler chickens, though alternative measures with beneficial features exist. Residual feed intake (RFI) is defined as the difference between observed feed intake (FI) and FI predicted from metabolic mid-weight and body weight gain. Efficient birds have negative RFI values (1). FCR is a ratio trait that has its mathematical limitations, whereas RFI is a linear index. RFI seems to be independent of production traits, and is moderately heritable. The aim of this study was to compare performance parameters pertaining to RFI and other feed efficiency traits (FCR, residual body gain (RBG), and residual intake and body gain (RIG)) in broiler chickens.

Methods: One-hundred-fifty-six 1-day-old Cobb 500 FF (Fast Feathering) chicks were used in three successive batches (n=26 females and n=26 males per batch). Chickens were individually housed in cages from day 7 to 35 of life. Chickens had free access to water and feed and were fed starter, grower and finisher diets from day 1-10, day 11-21, day 22-35, respectively. Body weight (BW) was measured weekly and feed consumption daily between day 7 and 35 of life. RFI and RBG were calculated as: RFI = FI [a + b_1 × $BW^{0.75}$ + b_2 × BWG] and RBG = BWG [a + b_1 × $BW^{0.75}$ + b_2 × FI], where a is the intercept and b_1 and b_2 are partial regression coefficients of FI, $BW^{0.75}$ and body weight gain (BWG), respectively. The FCR, RFI, RBG, and RIG were estimated from BW gain and feed intake data between day 7 and 35 of life using PROC REG of SAS. The top and bottom 15% of chickens, separately for males and females, were ranked as good and poor feed-efficient, respectively, whereas chickens in-between were considered as medium feed-efficient. Growth performance and feed intake data were compared among good, medium and poor feed efficient chickens according to RFI, RBG, RIG and FCR classification and were subjected to ANOVA using PROC MIXED of SAS. Moreover, regression analysis (PROC REG of SAS) was performed to correlate feed efficiency traits.

Results: The RFI of chickens linearly increased ($P < 0.001$) from low, medium to high RFI and differed by 388 g between low and high RFI chickens (Table 1). Total feed intake (TFI) linearly increased from low, medium to high RFI in female ($P = 0.001$) and male ($P < 0.036$) chickens. In contrast, total body weight gain (TBWG) was similar among chickens of different RFI. Separation of chickens according to their RBG resulted in a quadratic effect for TFI ($P = 0.044$) and TBWG ($P = 0.002$) in low, medium and high RBG male chickens, but not in females; with medium RBG males having the lowest TFI and TBWG. When chickens were separated according to their RIG, a linear effect for TFI ($P < 0.001$) was found similar to RFI classification, with increasing TFI from low, medium to high RIG. Likewise, ranking of chickens according to their FCR showed a linear increase ($P = 0.004$) from low, medium to high FCR in female chickens, and a linear increase ($P < 0.001$) in TBWG from low, medium to high FCR in male chickens. Regression analysis further demonstrated that RFI and RIG of chickens were highly negatively correlated ($R^2 = 0.98$; $P < 0.001$), whereas no relationship between RFI and RBG could be found. The RFI also positively correlated with FCR of chickens ($R^2 = 0.52$; $P < 0.001$); however, the relationship was weaker than with RIG.

	Least squares means ± SEM			Contrasts, P=	
	Low RFI	Medium RFI	High RFI	Linear	Square
Females					
RFI (g)	-195 ± 16.1	11.1 ± 9.66	193 ± 24.8		0.497
TFI (g)	3020 ± 68.5	3191 ± 41.3	3482 ± 106	0.001	0.422
TBWG (g)	2078 ± 42.2	2079 ± 25.7	2146 ± 65.4	0.388	0.482
Males					
RFI (g)	-234 ± 22.0	-30.8 ± 12.2	226 ± 24.6		0.205
TFI (g)	3416 ± 107	3466 ± 57.8	3904 ± 120	0.003	0.057
TBWG (g)	2460 ± 63.4	2389 ± 35.9	2506 ± 71.0	0.630	0.125

Table 1. Least-squares means (± SEM) for residual feed intake (RFI) and performance traits for female and male broiler chickens of groups ranked low, medium and high for residual feed intake during the experimental period from day 7 to 35 of life.[1]

[1]TFI, total feed intake; TBWG, total body weight gain.

Conclusions: Comparison of feed efficiency traits showed that results obtained for RFI and RIG classification of chickens were highly related, whereas results obtained for RBG and FCR differed from those obtained when chickens were ranked according to RFI. Present results support RFI as alternative measure to traditional ratio-type efficiency traits, such as FCR, in chickens, which can be used to investigate the underlying physiological and microbial factors causing the variation in feed-efficiency.

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40. Effect of silage type in ruminant rations on the abundance of different microbial groups in the rumen of dairy cows during a day

Effekt der Silage in Wiederkäuerrationen auf die Abundanz verschiedener Mikroorganismen im Pansen im Tagesverlauf

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Several *in vitro* studies showed that maize silage (MS) and grass silage (GS) differently affect different ruminal microbial groups [1, 2]. Similar *in vivo* comparisons by considering time effects are lacking so far. This study investigated effects of feeding MS and GS on numbers of different ruminal microbial groups during a day in dairy cows.

Methods: Two rations were fed to three ruminally fistulated lactating Jersey cows in three periods using an incomplete Latin Square design. The rations contained 52% concentrate (36% wheat, 36% barley, 14% soybean meal, 12% molasses, 2% mineral mix) and 48% GS or MS, respectively. Rations were offered for *ad libitum* intake for 20 days and 6 samples of the ruminal liquid and solid fraction (LF, SF) were taken on day 18 and 20 in 4-h intervals starting at 8:30 in the morning. Samples of the two days were pooled within sampling time. Microbial genomic DNA was extracted and partial sequences of mcrA, 18S and 16S rRNA genes were amplified by real-time quantitative PCR using primer sets specific for methanogens, protozoa, total bacteria, *P. bryantii* and *S. ruminantium*. They were expressed as gene copy numbers/ml LF or mg SF. Statistical analysis used a mixed model to account for the two factors 'silage' and 'sampling time' and the block structure.

Results: No effect of silage was detected for methanogens (data not shown). 18S rRNA gene copy numbers of protozoa were significantly higher for MS compared to GS in LF and SF ($P \leq 0.05$). Numbers of total bacteria in SF were also significantly higher for MS, but no silage effect was detected in LF (Fig.). The abundance of *P. bryantii* was higher with GS in LF and SF. Numbers of *S. ruminantium* also were higher with GS in LF, but not in SF. Sampling time had a significant effect on methanogens in LF and on protozoa and *P. bryantii* in both fractions. Lowest numbers of methanogens were found at 12:30 and 20:30, highest at 0:30 and 4:30. Numbers of *P. bryantii* decreased significantly between the first two sampling times in LF and SF. Thereafter numbers increased significantly between 16:30 and 20:30 as well as 20:30 and 0:30 in the LF and SF, respectively. For protozoa a significant interaction was detected between silage and sampling time. For GS the numbers decreased significantly between 8:30 and 12:30 in LF and between 20:30 and 0:30 in SF. For MS increasing protozoa numbers were found after 0:30 in LF, while numbers decreased significantly between 12:30 and 4:30 in SF.

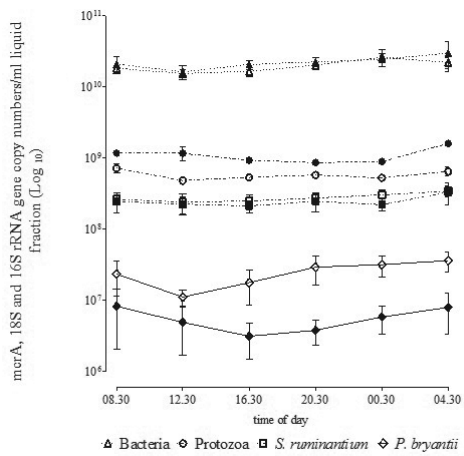

Fig.: mcrA, 18S and 16S rRNA gene copy numbers for microbial groups in liquid fraction (Mean, SEM; filled: maize silage; open: grass silage)

Conclusion: Ruminal microbes respond differently to MS and GS. MS caused higher abundance of total bacteria and protozoa probably due to higher starch concentration. Higher abundance of *S. ruminantium* and *P. bryantii* by GS feeding may have been caused by the higher crude protein concentration. Variable numbers of microbes at different day time suggest the need for repeated sampling in a day to obtain representative samples for describing diet effects on ruminal microbial communities.

[1] WITZIG et al. (2010): Anaerobe 16, 412-419.
[2] LENGOWSKI et al. (2014): Proc. Soc. Nutr. Physiol. 23, 49.

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41. Reticuloruminal pH dynamics during transient and persistent subacute ruminal acidosis in dairy cattle: a long-term evaluation with a wireless sensor

Reticuloruminale pH-Schwankungen während einer unterbrochenen und einer konstanten subakuten Pansenazidose bei Milchkühen: Eine Langzeit-Evaluierung mit kabellosen Sensoren

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Subacute ruminal acidosis (SARA) is a critical metabolic disorder of cattle, which is defined as extended periods of ruminal pH below 5.8. However, long-term studies investigating the severity of SARA, in particular with regards to their duration and persistency, are still missing. The study was aimed to evaluate ruminal pH changes and duration of pH drops in response to two SARA-challenge models, persistent and transient SARA challenge, during 41 d periods, using an indwelling wireless ruminal pH-sensor.

Methods: A feeding experiment was conducted with 8 rumen-cannulated Holstein cows which were assigned to a 2x 2 crossover design (2 SARA models and 2 experimental runs, n=8). The common feeding pattern was 6-d of 100% forage diet (DM basis), adaptation: 7-d gradual adaptation to the SARA diet (target level at 60% concentrate of diet DM), and at last, SARA: 28-d of SARA challenge. During the SARA period, one group of cows was subjected to the transient SARA challenge (SARA1) (7-d induction, 7-day break and 14-d re-induction) whereas the other group was in the persistent SARA model (SARA2) with no break. During the 7-d break cows of SARA1 were based on the 100% forage diet before returning to the SARA diet with a 2-d adaptation. To monitor ruminal pH continuously, all cows received a wireless ruminal pH-sensor (smaXtec animal care sales GmbH, Graz, Austria) via the ruminal cannula some days before the adaptation started, using a validated method (1). Data were analyzed following repeated measurement (days within each phase) in a 2 × 2 crossover design. The model included fixed effects of SARA model, feeding status (including baseline and adaptation) and their interaction, experimental run, and sequence (carryover effect). Cows were considered as random effect and nested within sequence. Statistical analysis of the rumen pH data was performed using SAS (SAS, version 9.2).

Results: Cows showed initial signs of SARA already during the adaptation with a pH < 5.8 over 3 h/d. Comparing SARA models, before the SARA-challenge (the first 13 d), as well as the first 7 d of SARA challenge, both models did not differ in pH (mean pH ranged from 6.08 - 6.42) and ruminal temperature (mean temperature ranged from 38.65 - 38.81 °C). The differences were more pronounced during the break in SARA1 ($P<0.05$), where pH of SARA1 was always above 6.00, while SARA2 still had lower pH (min = 5.49, max = 6.45 and mean = 6.15). In addition, the durations of pH < 5.5, 5.8 and 6.0 were ~8.5-, 6.5- and 3.5-fold longer in SARA2 than SARA1 ($P<0.05$). On the last 14 d of each SARA challenge, the opposite results were found as SARA1 caused a more pronounced decrease in pH and a prolonged time of low pH (e.g., mean pH = 5.88, and time < 5.8 = 518.9 min) than SARA2 (6.15, and 266.9 min respectively). Ruminal temperature of the last 14 d was also affected by SARA model as SARA2 had higher values than SARA1 ($P<0.05$).

Conclusions: Feeding 60% concentrate diet led to SARA conditions. However, the persistent challenge maintained the low ruminal pH, while exerting a 7-d break during the SARA challenge caused a more severe SARA condition during the second bout.

1. KLEVENHUSEN et al. (2014). J. Anim. Sci. 92:5635-5639.

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42. A study of the roles of bicarbonate and short chain fatty acids in ruminal buffering

Untersuchung über die Rolle von Bikarbonat und kurzkettigen Fettsäuren bei der Pufferung des Panseninhaltes

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Introduction: Clinically, diagnosis of ruminal acidosis continues to focus on ruminal pH, although in isolation, this parameter is an insufficient indicator of ruminal acid-base status. The absence of a stringent determination of the relevant constants for bicarbonate buffering in the rumen adds to the uncertainties involved. This study attempts to close this gap and introduces an easy to implement method for estimating relevant contributors to ruminal buffering.

Methods: After measuring intraruminal pH, ruminal fluid was obtained from a total of ten fistulated cows on different feeding regimes. Two of the animals were fed hay and increasing amounts of hay-silage (group 1) and sampled twice weekly for three weeks before and after meals, yielding a total of 24 samples. Eight samples were obtained from cows on a corn-hay-silage diet ad libitum with concentrate added on demand but limited by an automatic feeding system (group 2). Short chain fatty acids (SCFA) were measured using gas chromatography, while the solubility α of CO_2 and the pK_{app} of the bicarbonate system were studied at 38°C using the classical Astrup technique.

In an alternate approach, aliquots of the samples from group 1 were equilibrated at a pCO_2 of 5% and 100% (38°C). The total buffer value β_{tot}(pH) of the equilibrated aliquots was determined by titration with HCl and NaOH between pH 3 to 8, yielding two different buffer curves. Values for α, the pK_{app} and an estimate for the total SCFA in the sample $[SCFA]_{fit}$ were obtained using standard curve-fitting software (SigmaPlot) and the following relationship [1]:

$$\beta_{tot}(pH) = 2.302 \cdot \alpha \cdot pCO_2 \cdot 10^{(pH-pKapp)} + 2.302 \cdot [SCFA]_{fit}/(2+10^{(pH-pKSCFA)}+10^{(pKSCFA-pH)}) \quad (1)$$

Results: In group 1, mean ruminal pH as measured directly through the fistula was 6.55 ± 0.08 (n/N=24/2), significantly higher than the 5.52 ± 0.04, n/N=8/8 (p2of 5% or 100%, yielding values of 7.65 ± 0.04 or 6.41 ± 0.03, respectively (p

Using a variation of the Astrup technique, α in ruminal fluid was found to be 0.244 ± 0.01 $mmol \cdot l^{-1} \cdot kPa^{-1}$, while pK_{app} was 6.21 ± 0.03 (N/n=10/31). These values did not depend on the feeding group (p=0.43), the time after feeding (p=0.9), and showed no correlation to the SCFA concentration (Pearson coefficient P >0.05; $r^2 < 0.001$).

In the titration experiments, the contribution of SCFA to total buffer value dominated at pH < 6, and was maximal around a pH of ≈ 4.8. At pH values above 6, the contribution of the bicarbonate system rose exponentially in line with equation (1). Curve fitting after titration yielded α = 0.223 ± 0.003 $mmol \cdot l^{-1} \cdot kPa^{-1}$, pK_{app} = 6.05 ± 0.01(N/n=2/12) and $[SCFA]_{fit}$ = 81± 4 $mmol \cdot l^{-1}$, close to the mean value of $[SCFA]_{measured}$ = 80 ± 2 $mmol \cdot l^{-1}$ obtained via gas chromatography. Regression analysis yielded $[SCFA]_{fit}$ = -5.7 $mmol \cdot l^{-1}$ + 1.07 · $[SCFA]_{measured}$ (r^2 = 0.60)).

Conclusions: Ruminal buffering follows equation (1) with α ≈ 0.23 and pK_{app} ≈ 6.1. Accordingly, any spurious drop in the pCO_2 of samples of ruminal fluid can be expected to significantly affect measured pH. Establishing a generally accepted protocol with fixed levels of pCO_2 for measuring the acid-base status of ruminal samples should be considered.

1) Heisler N (1986): Acid-Base Regulation in Animals. Elsevier; Amsterdam: 3-48.

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43. Changes in body reserves with regard to energy- and protein intake and -yield in dairy cows fed barley-based diets treated with lactic acid and heat during early lactation

Energie- und Proteinaufnahme sowie die Leistung von Milchkühen im Hinblick auf ihre Körperreserven bei Fütterung von mit Milchsäure und Wärme behandelter Gerste in der Hochlaktation

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Feeding large amounts of grains is common in dairy cows during early lactation to enhance their energy intake as required in this period. Processing grains with organic acids was shown to influence their fermentation profile, as well as the DM intake (DMI) and milk composition of cows (1), with possible effects on their energy balance. The aim of this study was to compare the estimated energy- and protein balance of dairy cows fed processed or unprocessed barley over 100 d of early lactation.
Methods: After parturition, 30 dairy cows were offered a diet for ad libitum consumption, based on grass silage (15% DM basis), corn silage (20%), hay (15%), protein concentrate (11.5%), and barley (gradually increased to an amount of 38.5% at day 17 p.p.). On day 21 p.p., cows were randomly allocated to 3 feeding groups, receiving either a diet with unprocessed ground barley (C), or the same barley steeped in 1% lactic acid for 24 h at room temperature (LA), or in 1% lactic acid for 24 h in an oven at 55 °C (LA-H), until day 120 p.p. DMI, milk yield and milk composition, as well as body weight (BW) were measured daily and complete feed analyses performed monthly. The estimated balances of NEL and utilisable CP at the duodenum (uCP) were assessed on a weekly basis by comparing the individually measured intake to the individual milk yield and estimated losses (GfE, 2001). Body condition score (BCS) and back fat thickness (BFT) of each cow were evaluated 4 times (4.-6., 7.-10., 11.-13., and 14.-17. week p.p.) until day 120 p.p. Data were analysed by ANOVA using weekly intervals and treatment as fixed effects and accounting for repeated measures on time. Results one week before starting the experiment were used as a covariate in the model.
Results: The feed composition only showed small variations over time. Between week 5 and 7 p.p., DMI of the LA group was higher than that of the control ($p<0.05$), fat-corrected milk (FCM) yield and feed efficiency did not differ between groups ($p>0.05$). However, the estimated NEL- and uCP-balance were higher in LA and LA-H, than in the control group ($p<0.05$), with the major difference developing between week 4 and 7 p.p.. The estimated NEL-balance was around -15 MJ NEL 4 weeks p.p. and turned positive at week 6 (LA-H), 7 (LA) and 9 (C) p.p., respectively. BW was higher in the treatment groups ($p<0.05$), rising from 618 kg (± 9 kg) (all groups; week 4 p.p.) to 624 kg (C), 636 kg (LA) and 639 kg (LA-H), respectively (week 9 p.p.). As expected, the estimated NEL- and uCP-balance, as well as the BW, increased over time ($p<0.05$). The BCS showed a tendency towards higher values in both treatment groups ($p = 0.07$), but BFT did not differ among groups ($p>0.05$).
Conclusions: We previously reported that processing barley with LA and heat affected DMI (1) as well as the ruminal fermentation in terms of enhancing propionate formation and reducing butyrate (2). These changes plus the lack of an effect on FCM yield likely resulted in an improved NEL- and uCP-balance, leading to a faster recovery of the BW losses in the treatment groups p.p.. Further studies are warranted to characterize the underlying mechanisms and their practical implications.

(1) KHOL-PARISINI et al. (2014) Proc. Soc. Nutr. Physiol. (2014) 23: 6
(2) ZEBELI et al. (2014) Proc. Soc. Nutr. Physiol. (2014) 23: 67
(3) GfE (2001): Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder, DLG-Verlag, Frankfurt a. Main

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44. Digestibility of neutral and acid detergent fibre - a meta-analysis in dairy cows

Verdaulichkeit von Neutral und Säure-Detergenzienfaser – eine Metaanalyse bei Milchkühen

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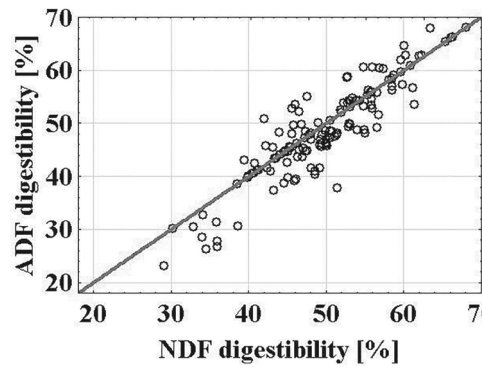
It is often assumed that digestibility of neutral detergent fibre (NDF) is generally higher than that of acid detergent fibre (ADF), but few actual evaluations exist. To test the relation of digestibility of the two fibre fractions, results from recent publications on NDF and ADF digestibility in dairy cows were evaluated and the impact of forage composition (proportion of maize, grass and legumes), forage to concentrate ratio (FCR) and intake level on fibre digestibility were investigated.

Methods: The data used for statistical evaluation were taken from the Journal of Dairy Science (years 2008 to 2014; Vol 91, Issue 1 - Vol 97, Issue 10). Data of all 36 studies available in this period were used, comprising 137 treatments (only studies with lactating dairy cows were used). Total collection of faeces was done in 11 studies, while 25 studies used markers to determine digestibility. Data were evaluated in R using a linear mixed model. Fibre fraction (NDF and ADF), forage composition (proportion of grass, maize, legumes and others [e.g. straw, whole crop silage]), FCR, dry matter intake (DMI), and their interactions (Table 1) were considered as fixed effects, the individual study was considered as random effect.

Results: Average fibre contents of diets [± standard deviation (SD)] were for NDF 33.9±4.01% DM and for ADF 21.2±3.30% DM. Reported NDF and ADF digestibility varied over a considerable range (Figure 1). On average, mean digestibility [± SD] of NDF was 49.6±7.67% and of ADF 48.5±8.99%. In 21% of the evaluated cases, ADF digestibility was below the range of mean NDF digestibility minus 1 SD, while in 15% of the cases, ADF digestibility was higher than mean NDF digestibility plus 1 SD. In the linear model, NDF was not found to be different from ADF digestibility (Table 1). No general effects of DMI, forage composition and FCR were found. Only the interaction DMI × fibre fraction was significant.

Table 1. P-values for the fixed effects.

Fibre fraction (NDF or ADF)	0.284
DMI	0.129
Grass	0.690
Maize	0.435
Legumes	0.598
Others	0.211
FCR	0.670
DMI × fibre fraction	0.003
FCR × fibre fraction	0.915
Grass × fibre fraction	0.941
Maize × fibre fraction	0.517
Legumes × fibre fraction	0.994
Others × fibre fraction	0.685



Conclusions: Lignin as a major indigestible proportion of the diet is inherently higher in ADF than in NDF, so a lower digestibility of ADF compared to NDF could be expected. However, from the present results it can be concluded that digestibility of ADF must not be considered to be generally lower than that of NDF. Apparently, linkages with lignin render higher proportions of hemicellulose than cellulose indigestible, thus, balancing the aforementioned effect of the higher lignin proportion of ADF. This scenario is in line with the result that DMI influenced ADF digestibility more than that of NDF (significant interaction DMI× fibre fraction).

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45. Effects of feeding different energy supply on the microbiota attached to the rumen wall in goats

Langfristige Effekte unterschiedlicher Energieversorgung auf die pansenwandassoziierte Mikrobiota bei der Ziege

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Question: Ecological balance in the rumen is highly sensitive to energy-rich diets. Yet the effects of energy-rich feeding on the bacteria attached to the rumen wall, a microbial community with important physiological functions in the rumen, are largely unexplored. This study aimed to investigate the impact of dietary energy supply on the epimural bacteria in the rumen of goats using deep sequencing methods.

Methods: Seventeen growing goats were fed low (n = 5; 6.2 MJ ME/d; 0% concentrate), medium (n = 6; 7.3 MJ ME/d; 30% concentrate) or high (n = 6; 10.2 MJ ME/d; 60% concentrate) dietary energy supply. Chopped meadow hay (second cut) was fed and coarsely ground barley grain was used as concentrate. Goats were transitioned to the 30 and 60% concentrate diets by gradually increasing the dietary grain level by 2.5 and 5% per day, respectively. Goats were euthanized 2 hours after their last feeding, and tissue samples of the cranial part of the ventral rumen were collected and used for microbial analysis. The pH of rumen fluid was measured immediately, whereas DNA was isolated from thawed rumen mucosa and used for Roche/454 16S rRNA gene amplicon pyrosequencing, yielding 122,458 reads. Pyrosequencing data were clustered into 1,879 operational taxonomic units (OTU, 0.03 distance level). Additionally, near full-length 16S rRNA gene cloning and sequencing (n = 328) was performed. 179 pyrosequencing OTUs showed ≥ 99% similarity with full-length clones. Analysis of the sequence data was done with the software package mother. To illustrate microbial shifts on community level, discriminant analysis was done in JMP Pro (SAS). ANOVA was performed with Proc Mixed of SAS accounting for fixed effect of energy supply and the random effect of animal.

Results: Pyrosequencing revealed *Proteobacteria, Bacteroidetes, Firmicutes* and *Spirochaetes* being the most abundant phyla (97.7%). Compared to the medium group, the high and low energy groups harbored significantly more *Firmicutes* (1.75 fold-change), and *SR1* (5.54 fold-change), respectively ($P < 0.05$). A rarely detected genus in ruminal samples, *Bergeriella*, was highly abundant (21%) in all groups. On OTU level a *Bergeriella*-related OTU was most abundant (14%) in the epimural bacterial community, followed by two *Campylobacter* OTUs, which responded differently to diets: OTU 2 was significantly increased (2.89 fold-change) whereas OTU 3 was significantly decreased (0.25 fold-change) with highest energy supply. At the genus level, goats fed low energy diet tended to have less Ottowia (0.21 fold-change, $P < 0.07$) and had less *Mogibacterium* (0.24 fold-change, $P < 0.03$), whereas they had increased *Kingella* (5.96 fold-change, $P < 0.04$) compared to goats of the other feeding groups. Furthermore, goats fed the low energy diet tended to have more *Bergeriella* than goats fed the medium energy diet (2.1 fold-change, $P < 0.08$). The genus *Bergeriella* was significantly decreased (0.22 fold-change, $P < 0.02$) in the high energy group compared to the low and medium energy diets. Feeding of high energy diet also tended to increase *Prevotella* compared to goats fed the other two diets (1.66 fold-change, $P = 0.07$). Ruminal pH of goats fed the high energy diet (5.5) was lower ($P < 0.01$) than low (6.4) and medium (6.0) feeding groups.

Conclusions: The study describes the core bacterial community attached to the rumen wall in goats. High energy feeding influenced the composition of the epimural bacteria, mainly by lowering fiber-digesting bacteria and enhancing starch-fermenting bacteria. The results of the study provide novel insights into the adaptive changes in the core rumen epimural bacteria relative to high dietary energy supply.

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46. Effects of treating barley grain with citric or lactic acid with or without inorganic phosphorus supplementation on *in vitro* fermentation characteristics

Effekte einer Behandlung von Gerste mit Zitronen- oder Milchsäure mit und ohne Phosphor Ergänzung auf die ruminale Fermentation in vitro

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Question: Chemical processing of cereal grains with mild organic acids has been suggested to modulate their chemical composition and starch type. Treating barley grain with LA reduced the myo-inositol hexakisphosphate concentration of cereals (1). We evaluated the effects of treating barley grain with lactic (LA) or citric acid (CA), with or without inorganic P supplementation, on *in vitro* ruminal fermentation characteristics, using the rumen simulation technique (RUSITEC).

Methods: Samples of 50 g of crushed barley grain "*Espinosa*" were incubated with 60 ml of either 5% LA, 5% CA or deionized water (control) for 24 h and then dried for 72 h at room temperature (20 - 22 °C). Six different mixed diets were formulated by combining each barley treatment (LA, CA, control) with two different mineral premixes (either with 36.43 g P/kg DM (monocalcium phosphate) or without P). The dietary components (48.9% hay, 10% soybean meal, 40% barley grain, 1.1% mineral-vitamin premix with P or without P) were ground to a theoretical particle size of 2 mm and incubated with rumen innocula of dairy cows. The P-restricted diet contained 3.08 g native P/kg DM, whereas the diet supplemented with P contained 3.63 g P/kg DM, with the mineral premix contributing for 0.55 g P/kg DM as monocalcium phosphate. A 12-fermenter RUSITEC system was used in 3 randomized 10-day runs (n = 6 per treatment), each run including a five day adaption- and a five day sampling-period. The fermenters for the diets with supplemental inorganic P were run using the common McDougall's buffer solution that contains phosphates (0.87 g P per fermenter and day). To run the six fermenters free of inorganic P, a modified McDougall buffer solution without P was used. During the sampling period, rumen fluid from each fermenter was collected daily before feeding. Short-chain fatty acids were analyzed by gas chromatography, and bacterial groups were quantified by quantitative PCR. An analysis of variance was performed with SAS (9.2) to test the fixed effects of grain treatment, inorganic P supplementation, and their two-way interactions, using a model for repeated measures.

Results: Compared to the native control (49.3%), CA treatment significantly increased acetate concentration (50.8%; $P = 0.0116$) and LA treatment significantly decreased acetate concentration (45.8%; $P < 0.001$). In diets with P-supplementation, acetate was decreased from 50.4% to 46.8% ($P < 0.001$) by LA treatment. Compared to the native control (24.4%), propionate was significantly increased (29.5%; $P = 0.0047$) by LA treatment. Compared to the control with P-supplementation (21.7%) propionate was increased by CA (25.5%; $P = 0.0017$) as well as LA (28.3%; $P = 0.0045$) treatment. *Ruminococcus albus* (0.004% vs. 0.005% of total bacteria; $P = 0.0178$) and genus *Prevotella* (23.3% vs. 26.7%; $P = 0.0064$) significantly increased by P supplementation. *Ruminobacter amylophilus* (0.01% vs. 0.008%; $P = 0.0189$) and *Megasphaera elsdenii* (1.7% vs. 1.1%; $P = 0.0406$) were significantly decreased by P supplementation. Treatments with CA (2.3%; $P = 0.0026$) and LA (2.7%; $P = 0.0002$) increased the amount of *Clostridium cluster* XIV compared to the control (1.7%). Anaerobic fungi were increased by LA treatment (4.8% vs. 4.9%; $P = 0.0101$).

Conclusions: The data showed that treatment of barley with CA and LA lowers acetate:propionate ratio. Acid treatments of barley and P supplementation affected microbial composition such as important amylolytic and fibrolytic bacteria and lactate utilizers.

(1) METZLER-ZEBELI et al. (2014) PLoS ONE 9(6): e101166. DOI 10.1371

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47. *In situ* and total tract nutrient degradation in dairy cows fed barley-based diets treated with lactic acid and heat

In situ- und Gesamttraktverdaulichkeit der Nährstoffe von Milchkühen bei Fütterung von mit Milchsäure und Wärme behandelter Gerste

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Dairy cows are fed large amounts of grains to enhance their energy intake. This feeding procedure increases the risk of rumen disorders, which may result in lowered nutrient digestibility. Processing of grains with lactic acid (LA) has been shown to affect starch degradation in vitro (1), but no data are available on the effects of treatment on rumen degradation of starch and digestibility of dietary nutrients. The aim of this study was to evaluate effects of barley grain treatment with LA or LA plus heat on in situ degradation and total tract digestibility of nutrients in dairy cows.

Methods: Six Holstein dairy cows, fitted with a permanent ruminal cannula (i.d. 100 mm), were used in a double 3 × 3 Latin square design. Cows were offered 3 diets for ad libitum intake consisting of approximately 39% ground barley grain (CON) or the same amounts of ground barley grain steeped for 24h in 1% LA at room temperature or in an oven at 55°C (LAH). Each of 3 experimental periods lasted 21 d with the first 14 d used for diet adaptation. Diets also contained (DM basis) on average 13% grass silage, 22% maize silage, 15% hay, and 11% protein, mineral and vitamin mixture. Nylon bag technique was used to determine degradation of all nutrients of barley including that of starch. For this, untreated or treated barley samples were incubated up to 48h in the rumen of cows consuming one of the respective diets. TiO_2 was used an external marker to estimate total tract digestibility during the last 7 d of each experimental run. Kinetics variables were determined by PROC NLIN, whereas ANOVA was performed with PROC MIXED of SAS (9.2), using a significance and trend level of $P < 0.05$ or $P < 0.10$, respectively.

Results: Feed intake of cows was not different ($P > 0.05$) among diets (17.8, 18.7, 18.3 kg/d for CON, LA, and LAH, respectively). While no difference was observed at 0h, at 2h after incubation the processing with LA and LAH lowered ($P < 0.05$) in situ degradation of barley starch from 45.5 to 39.6 and 34.5% (SEM = 2.52) for CON, LA and LAH, respectively. The difference became even greater at 4h after incubation (decrease from 62.6 to 53.8 and 53.6%; SEM = 3.01), but minimized thereafter. Kinetics data showed a tendency for LAH treatment to lower overall degradation rate of starch from 22.6 to 17.2 and 15.1%/h; $P = 0.07$). Degradation of barley CP was higher at 0h for LAH treatment (17.1, 15.9, 23.0%; $P = 0.01$) but tended to decrease at 4 h (51.8, 45.6, 48.1%, $P = 0.09$). Disappearance of ash was greater ($P < 0.01$) with LA (58.6%) and LAH (62.0%) treatment compared to CON (36.7%). No significant effect of treatment on in situ DM degradation of barley grain was observed. Total tract digestibility of the whole diet DM was greater ($P < 0.05$) in cows fed LA (66.6%) and LAH (67.4%) than the control (65.0%) diet. However, the digestibilities of other nutrients and the intake of digestible OM were not different among diets ($P > 0.05$).

Conclusions: Processing barley with LA and LAH lowered degradation of starch by more than 10% at 2 and 4 h after incubation, suggesting an increment of rumen undegradable starch in treated barleys. Enhancement in the disappearance of ashes indicates an improved availability of barley minerals by LA treatment. The increase of total tract DM digestibility did not result in an improvement of digestible OM intake of cows.

(1) DECKARDT et al. (2014) Starch/Stärke. 66: 558-565.

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48. Continuous recording of pH in the forestomach of dairy cows using two different measurement systems

Aufzeichnung des pH-Wertes im Vormagen von Milchkühen mit zwei verschiedenen Messsystemen

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Question: Subacute ruminal acidosis (SARA) is one of the most important digestive disorders in high yielding dairy cows fed highly fermentable diets. SARA is insidious and its clinical signs are difficult to detect. Continuous monitoring of the ruminal pH may prove to be a valuable tool for this purpose (1). The aim of the present study was to compare continuously recorded measurements of an indwelling telemetric pH probe placed orally in the reticulum with those obtained from a measurement system placed in the ventral part of the rumen through a cannula.

Methods: The experiment was conducted with 6 ruminally cannulated Holstein cows kept in a free-stall barn. The cows were equally distributed into 2 treatment groups based on their previous lactation performance. Cows in treatment CON- were offered a diet consisting of only roughage and cows in treatment CON+ got roughage and were supplemented with a cereal-based and a corn gluten-based concentrate to meet their predicted nutrient requirements. The experiment lasted from 2 wk before the predicted calving date until wk 8 of lactation. During the whole experiment the pH was measured in the reticulum every 10 min with a telemetric pH-Bolus (eBolus, eCow Ltd, Exeter, Devon, UK) which had been applied orally using a balling gun. Furthermore, in wk 2 before the estimated calving date and in wk 2, 4, 6, and 8 of lactation ruminal pH was measured additionally every 30 sec during 48 h with the LRCpH measurement system (DASCOR Inc., Escondido, CA, USA) which was placed in the rumen through the cannula. For the statistical analysis the readings of the LRCpH measurement system were summarised over 10 min. In order to compare the mean pH of both measurement systems a linear mixed model was set up with measurement system, treatment and wk of lactation as fixed effects. The same model was used to determine the effect of treatment and wk of lactation on the pH difference (ΔpH) between measurement systems.

Results: In general, the pH profiles recorded with the eBolus showed less fluctuations than the profiles recorded with the LRCpH measurement system. The mean pH measured with the eBolus was higher compared to those measured with the LRCpH. The difference was 0.24 pH units ($P < 0.001$). The mean pH was not influenced ($P > 0.05$) by treatment but it decreased continually from 2 wk before calving until wk 4 of lactation ($P < 0.001$) whereupon it remained stable ($P > 0.05$) until wk 8 of lactation. The treatment had no effect ($P > 0.05$) on the ΔpH between measurement systems. However, the ΔpH was different ($P < 0.01$) in wk 2 compared to wk 4 and to wk 8.

Conclusion: The pH profile measured with the eBolus in the reticulum seems to fluctuate less than the pH measured with the LRCpH measurement system in the rumen. Furthermore, the mean pH measured in the reticulum was higher compared to the pH in the rumen. However, due to fluctuation in the ΔpH across wk of lactation no fixed correction factor can be provided.

1) GOZHO, G.N., PLAIZIER, J.C., KRAUSE, D.O., KENNEDY, A.D., WITTENBERG, K.M. (2005): J. Dairy Sci. 88: 1399-1403

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49. Effects of supplementing maize silage with different nitrogen sources on ruminal fermentation *in vitro*

Effekte einer Ergänzung von Maissilage mit verschiedenen N-Quellen auf die Pansenfermentation in vitro

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Previous *in vitro* studies showed an improved degradation of organic matter (OM) and efficiency of microbial protein synthesis (EMPS) when maize silage (MS) was partially replaced by high nitrogenous grass silage (GS) [1]. Urea supplementation of MS increased OM degradation and EMPS, but not at the level achieved with GS [2]. The present study aimed to investigate effects of different nitrogen sources supplemented to MS on fermentation characteristics *in vitro*.

Methods: A semi-continuous rumen simulation technique (Rusitec) with 5 vessels per treatment across 5 experimental runs (n=5) was used. Oven-dried MS and GS (7 and 14% CP on dry matter basis, respectively) were ground through a 1-mm sieve, filled into nylon bags (15 g), and incubated. MS remained either unsupplemented or was supplemented with urea, pea peptone, pea protein or amino acids (AA, mixed in accordance to the AA profile of the pea protein) to adjust N contents to that of the GS. Each Rusitec run lasted for 13 d and individual bags were incubated for 48 h. The artificial saliva contained $^{15}NH_4Cl$ for calculation of microbial CP synthesis. Residues from nylon bags were sampled and pooled within a vessel from d 7-12 and analysed for crude nutrients, NDF and ADF. Solid associated microbes were isolated on d 13 and considered for calculation of degraded OM, CP, and microbial CP synthesis of this fraction. Liquid associated microbes were isolated, pooled per vessel (d 7-13) and analysed for ^{15}N concentration to calculate microbial CP synthesis in fermenter liquids. Short chain fatty acids (SCFA) were analysed in fermenter effluents. A mixed model was fitted to test the fixed treatment factor. It accounted for block effects, 'run' and 'fermenter'.

Results: Degradation of OM was lowest for MS and MS+Protein, highest with the other N supplements and intermediate for GS (Table). Similar results were found for CP degradation except for MS showing a higher value compared to MS+Protein and GS ($P \leq 0.05$). The highest degradation of fibre fractions was detected for GS. NDF degradation was increased by AA and peptone whereas ADF degradation was increased by urea, peptone and protein. Production of valerate and iso-SCFA was highest for MS supplemented with AA or peptone indicating a high AA fermentation. Isobutyrate production was also high for GS. The EMPS was significantly increased by all N supplements but achieved the level of GS only in the case of peptone supplementation.

Table: Degradation of crude nutrients, fibre fractions (%), production of SCFA (mmol/d) and EMPS (g microbial CP/kg degraded OM) (LS Means, *SE*)

	MS	MS+Urea	MS+AA	MS+Peptone	MS+Protein	GS	***P*** value
OM[1)]	40^{c} (0.5)	45^{a} (0.5)	45^{a} (0.5)	44^{a} (0.5)	40^{c} (0.5)	42^{b} (0.5)	***
CP[1)]	59^{d} (1.0)	76^{a} (1.0)	66^{c} (1.0)	69^{b} (1.0)	37^{f} (1.0)	55^{e} (1.0)	***
NDF_{OM}	11^{c} (0.5)	11^{c} (0.5)	13^{b} (0.5)	13^{b} (0.5)	11^{c} (0.5)	19^{a} (0.5)	***
ADF	5^{d} (0.5)	9^{b} (0.5)	6^{cd} (0,6)	7^{c} (0.5)	7^{c} (0.5)	19^{a} (0.5)	***
Isobutyrate	0.25^{d} (0.01)	0.23^{e} (0.01)	0.45^{a} (0.01)	0.41^{b} (0.01)	0.25^{d} (0.01)	0.37^{c} (0.01)	***
Isovalerate	1.48^{bc} (0.20)	1.50^{bc} (0.20)	1.93^{a} (0.20)	1.68^{ab} (0.19)	1.37^{c} (0.19)	1.28^{c} (0.19)	***
Valerate	2.24^{b} (0.16)	2.79^{b} (0.16)	3.20^{a} (0.16)	3.21^{a} (0.16)	2.95^{ab} (0.16)	2.76^{b} (0.16)	**
EMPS	111^{d} (2.6)	183^{b} (2.6)	183^{b} (2.6)	196^{a} (2.6)	147^{c} (2.6)	200^{a} (2.6)	***

[1)] Corrected for contribution of microbial mass attached to feed residues; P value for F-test of treatment; [a-f] Identical super-scripts within a row indicate non-significant differences of LS Means between treatments ($P \leq 0.05$); **$P < 0.01$; ***$P < 0.001$

Conclusion: The improvement of OM degradation and EMPS by adding urea to MS supported previous findings [1] and was similar with AA addition. However, EMPS similar to GS was only achievable when peptone was supplemented to MS. Probably this was linked to AA fermentation being somewhat lower with peptone compared to AA supplement. The low increase of EMPS with pea protein supplementation probably was related to the very low degradation of the pea protein.

[1] HILDEBRAND et al. (2011a): Animal 5, 528-536.
[2] HILDEBRAND et al. (2011b): Arch. Tierernahr. 65, 402-414.

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50.

Feed intake, chewing behaviour and digestibility in dairy cows of different age fed Swiss standard or zero-concentrate diets

Futteraufnahme, Kauverhalten und Verdaulichkeit bei verschieden alten Milchkühen mit Rationen mit oder ohne Kraftfutter

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Milk yield of dairy cows increased remarkably in dairy production systems in the last decades by various breeding, management and feeding measures inclusive of the use of high-concentrate diets. However, this intensification is often associated with a decreased lifetime of the cows for instance due to culling for fertility reasons or to increase herd milk yield. As an alternative to such a high-input/high-output strategy, a low-input/moderate-output strategy could be pursued which aims at maximising lifetime performance and longevity, and at minimising the use of concentrate. The success of such an approach requires that there are no adverse changes in physiological parameters with age. As a first step, feed intake, chewing behaviour and digestibility were determined in cows of different age fed either on a Swiss standard or a zero-concentrate diet.

Methods: For the experiment, 2×15 Brown Swiss dairy cows in their 1st to 7th lactation were selected from the herd of the Agricultural Education and Advisory Centre Plantahof, Landquart (CH). This herd had been split into two sub-herds in 2003. One sub-herd continued to receive a standard diet (forage at *ad libitum* access plus 5 kg/d concentrate) while the other sub-herd never gets concentrate. The forages offered in the experiment were hay, maize silage and grass pellets. Measurements were conducted during 8 days in a tie stall barn at Plantahof. Chewing activity was recorded with pressure sensors mounted on the noseband of halters (1) on 4 of the 8 days. The times the animals spent eating, ruminating and idling were identified. Analysis of variance was performed with diet and age as factors and energy-corrected milk yield and body weight as continuous variables. For that purpose, cows were grouped into primiparous (n=7), young (2nd to 4th lactation, n=11) and old cows (>4th lactation, n=12). In addition, age effects were also studied by regression analysis with age in days as continuous variable.

Results: Dry matter intake (DMI) did not differ (P=0.38) between sub-herds, but, due to the omission of concentrate, the zero-concentrate group ate more (P<0.01) neutral detergent fibre (NDF). Old cows had a higher (P<0.01) DMI than primiparous cows, but there was no difference (P=0.51) between young and old cows. No significant differences (P=0.25) between old and young cows were found for NDF intake, either. Absolute times of eating and ruminating did not differ between sub-herds or age classes. However, relative to DMI, old cows had a shorter (P=0.03) rumination time than primiparous cows, whereas this was similar (P=0.99) between old and young cows and also between sub-herds (P=0.20). Cows receiving no concentrate spent less (P=0.04) time ruminating per kg NDF than standard fed cows. Primiparous cows spent more time eating per kg DMI (P=0.01) and NDF (P=0.03) than old cows. Regressions showed an initial increase in organic matter (OM) digestibility with age, which levelled out from around the 3rd lactation onwards. The OM digestibility of the diet supplemented with concentrate was higher (P<0.01) by 2.6%-units.

Conclusion: As expected, primiparous cows had a lower DMI than multiparous cows, but no significant differences between old and young cows were found. There were some age-related differences in eating and rumination behaviour indicating an optimisation with age, but this resulted in a higher OM digestibility only for some years. Differences between diet types were less pronounced than expected, probably due to the limited use of concentrate on the one hand and the substantial use of highly digestible grass pellets in the zero-concentrate diet.

1) NYDEGGER F., GYGAX L., EGLI W. (2011): Agrarforsch. Schweiz. 2:60-65.

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51. Establishing an *in vitro* model to evaluate the effects of subacute ruminal acidosis on rumen microbial ecology in cattle

Etablierung eines in vitro Models zur Evalulierung der Auswirkungen einer subakuten Pansenazidose auf die mikrobielle Ökologie im Pansen beim Rind

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Subacute rumen acidosis (SARA) is a common concern in dairy cattle. SARA may result in major ruminal microbial alterations and accumulation of bacterial endotoxins with severe consequences for the host´s health (1). Microbial alterations due to SARA have been well characterized in cattle (2). Yet, *in vivo* measurements of microbial activity under SARA conditions are stressful for the animal and can severely impair the animal´s health. Hence, alternative *in vitro* procedures could be a useful tool for initial investigations of ruminal microbial changes and their consequences for ruminal fermentation in relation to SARA. The study aimed to establish an *in vitro* model for SARA using rumen simulation technique (RUSITEC) to evaluate changes in rumen microbial ecology.

Methods: A RUSITEC-experiment with 12 fermenters was conducted using rumen innocula from 3 different donor cows. Two incubation conditions with 6 fermenters each were established including NORMAL (target ruminal pH ~6.7) and SARA (pH ~5.5) by adjusting diet composition and concentration of McDougall's artificial saliva. For NORMAL conditions, the diet consisted of 50% concentrate and 50% forage (DM basis), whereas the dietary concentrate level of SARA conditions was enhanced to 65% (DM basis), and the McDougall buffer was modified by decreasing the concentrations of $NaHCO_3$ from 117 mM to 50 mM and of $Na_2HPO_4 \times 2\ H_2O$ from 26 mM to 10 mM. The experiment consisted of 3 runs, each lasting for 12 d, resulting in 18 replicates (n=18). The last 5-d of incubation liquid samples of each fermenter unit were pooled (from each fermenter over 5-d) before microbial analysis. Target microbes were quantified using quantitative PCR. Another portion of the samples was analyzed for bacterial endotoxin using *Limulus* Amebocyte Lysate test. Data were subjected to analysis of variance by MIXED procedure of SAS.

Results: Throughout the experiment the pH was maintained at a range of 5.6-5.8 for SARA conditions, being similar to *in vivo* SARA conditions, whereas NORMAL pH ranged between 6.5-6.8. Compared to Control, SARA increased ($P < 0.001$) total bacterial gene copy number (log_{10}/mL), 9.61 vs. 9.25; ± 0.03. SARA greatly reduced the proportion (% of total bacteria) of *Ruminococcus albus* and *Fibrobacter succinogenes* ($P < 0.001$) which are the major ruminal fibrolytic bacteria, respectively). On the other hand, SARA increased ($P < 0.05$) bacterial groups that include amylolytic bacteria such as genus *Prevotella and Lactobacillus* group. Lactate utilizers such as *Megasphaera elsdenii* and *Selenomonas ruminantium* were dramatically increased under SARA condition ($P < 0.001$). Total protozoa gene copy number (log_{10}/mL) was decreased during SARA (4.20 vs. 6.47; ± 0.12), but with a greater increase in *Entodinium spp* proportion (1.7 times), while total methanogens abundance was higher compared to NORMAL ($P < 0.05$). Endotoxin level was increased during SARA (59.65 vs. 36.08 EU/mL; ± 4.45) compared to NORMAL rumen conditions ($P < 0.05$).

Conclusions: An *in vitro* SARA model was successfully developed for cattle over a period of 12 days. The major changes in ruminal microbiota obtained during this *in vitro* SARA study are comparable to the aforementioned signature changes observed *in vivo* SARA conditions. Hence, this *in vitro* SARA model could be an initial tool for future studies, especially for those dealing with screening unknown feed additives to mitigate SARA and its associated alterations in cattle.

(1) ZEBELI and METZLER- ZEBELI (2012) Res. Vet. Sci. 93: 1099-1108.
(2) CHEN et al. (2011) Appl. Environ. Microbiol. 77: 5770-5781.

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52. Effects of enzymatically modified starch product on serum lipids in growing pigs

Einfluss eines enzymatisch modifizierten Stärkeprodukts auf das Serumlipid-Metabolom beim wachsenden Schwein

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Consumption of resistant starch (RS), being indigestible to mammalian enzymes, has proven beneficial to improve blood glucose and insulin levels (1). Evidence is growing that RS can also lead to alterations in systemic fat metabolism. Chemically modified starches (CMS) have become popular in food science as the chemical modification of the starch molecule allows reducing the digestibility of starch to a defined extent. The underlying physiological effects of CMS have been, however, less well explored than those of naturally occurring RS (1). The aim of this study was to evaluate the effects of an enzymatically modified starch product (EMS) on serum lipid profiles in the pre- and postprandial phase using catheterized pigs.

Methods: Castrated male pigs (n=8; 38 kg) were surgically fitted with a jugular-vein catheter and were fed semi-purified diets based on cornstarch and casein. The dietary starch component was either the control starch or the EMS. After an 8-day adaptation to the diets and a fasting period of 15-hours, a meal tolerance test was performed with serial blood samplings from -30 to 480 min postprandial. Blood samples for metabolomics were collected at -30 and 60 min postprandial. Serum triglycerides, cholesterol, non-esterified fatty acids (NEFA) were determined by standard enzymatic colorimetric analysis. Serum metabolomics was performed using a targeted metabolomics approach based on electrospray ionization liquid chromatography-mass spectrometry. Data were subjected to ANOVA using PROC MIXED of SAS; considering time-repeated measures on the same pig.

Results: Pigs fed the EMS diet had similar fasting concentrations of triglycerides (control diet, 0.28 mmol/l vs. EMS diet, 0.25 mmol/l (SEM ±0.024)) and cholesterol (control diet, 2.01 mmol/l vs. EMS diet, 2.14 mmol/l (SEM ±0.113)) compared to pigs fed the control diet; however, EMS consumption led to a 37% increased fasting NEFA concentration ($P = 0.016$) compared to the control diet (control diet, 155 µmol/l vs. EMS diet, 213 µmol/l (SEM ±16.7)). Serum triglycerides and NEFA decreased during the first 60 min after feeding ($P < 0.05$) and triglycerides started to increase again from 150 min and 360 min postprandial in pigs fed the control diet and EMS diet, respectively. Serum triglycerides were reduced in pigs fed the EMS diet at 180 and 360 min and, as trends ($P < 0.1$), at 30, 210, 240 and 300 min postprandial compared to pigs fed the control diet, causing a 23% lower area-under-the-curve with EMS diet ($P < 0.1$; control diet, 108 mmol/l × 480min vs. EMS diet, 84 mmol/l x 480 min (SEM ±8.4)). Serum NEFA were similar for both diets until 480 min postprandial when the EMS diet tended ($P < 0.1$) to cause a 40%-higher NEFA concentration compared to the control diet. Consumption of EMS altered the serum phospholipid metabolome with similarly directed alterations pre- and postprandially. Consumption of EMS reduced serum concentration of several long-chain sphingomyelins, increased many serum lysophosphatidylcholines and either increased or decreased phosphatidylcholines compared to the control diet.

Conclusions: Present results demonstrated that EMS has the ability to attenuate postprandial raise in serum lipids and may increase lipolysis in the fasting state, thereby supporting that feeding of RS lead to alterations in systemic fat metabolism.

(1) Birt et al. (2013) Adv Nutr 4: 587-601.

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53. Alterations in the cecal bacterial metagenome in response to resistant starch type 4 in growing pigs

Einfluss von resistenter Stärke Typ 4 auf das bakterielle Metagenom im Zäkum beim wachsenden Schwein

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Resistant starch (RS) includes all starch and starch degradation products that are not digested by host enzymes in the small intestine (1). Though resistant to host digestion, bacterial species that reside in the gastrointestinal tract are capable of utilizing RS as a substrate. Consequently, alterations in the composition of the gut microbiota in response to RS consumption were reported including enrichment of bacteria related to bifidobacteria, *Bacteroides, Eubacterium* and *Ruminococcus* as well as decrease in enterobacteria (2). Most information was gained using RS type 2 and 3, whereas RS type 4 is less well explored. In the present study, we therefore aimed at assessing the effect of resistant starch type 4 on the cecal bacterial metagenome in growing pigs.

Methods: Castrated male pigs (n=24; 29 kg) were were fed semi-purified diets based on cornstarch and casein. The dietary starch component was either the control starch or the resistant starch type 4 (enzymatically modified starch (EMS)). After a 10-day adaptation to the diets, cecal digesta samples were collected on day 11 three to four hours after the last feeding. Genomic DNA isolated from cecal samples were used for sequencing on a MiSeq Illumina Platform targeting the 16S rRNA gene. Sequencing data were analyzed using MOTHUR software and assignments of sequences to OTUs were based on a cutoff of 97% sequence similarity. Data were subjected to ANOVA using PROC MIXED of SAS, multivariate analysis using JMP 10, and k-means clustering using Cluster 3.0.

Results: Of the 2762 assigned OTUs, 1374 OTUs were abundant in pigs fed the EMS diet and control diet, whereas 547 OTUs were unique for pigs fed the EMS diet and 841 OTUs could be only found in pigs fed the control diet. Chao 1, Shannon diversity and Simpson index indicated similar richness and diversity of bacterial communities in pigs fed the EMS and control diet. Linear discriminant analysis showed clearly distinguishable cecal community profiles for the EMS and control diets. Clustering analysis also supported varying community profiles for the two diets. Overall, there was a big variation in OTU abundance among individual pigs, rendering it difficult to distinguish EMS-related effects on OTU level. Overall, pigs fed the EMS diet harbored 10%-less OTUs related to Firmicutes ($P = 0.09$) and were colonized 10%-more Proteobacteria compared to pigs fed the control diet. Cecal abundance of *Ruminococcus*, *Parasutterella*, *Bilophila and Enterococcus-like OTUs* ($P < 0.05$) and, as trend ($P < 0.1$), of *Lactobacillus-like* OTU decreased with EMS diet, whereas OTUs being closely related to *Meniscus* and *Actinobacillus* ($P < 0.05$) and, as trend ($P < 0.1$), of *Campylobacter* and *Oscillibacter* were increased in cecal digesta of pigs fed the EMS diet compared to those fed the control diet.

Conclusions: Present results showed that consumption of EMS led to characteristic shifts in the cecal bacterial community profile of growing pigs; however, effects were different compared to previously reported effects related to RS type 2 and 3.

1) Birt et al. (2013) Adv Nutr 4: 587-601.
2) Martinez et al. (2011) PLoS ONE 5(11).

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54. Expression of pro-inflammatory NF-κB and anti-oxidative Nrf2 target genes in different parts of the intestine of pigs between weaning and day 21 post-weaning

Expression von pro-inflammatorischen NF-κB und anti-oxidativen Nrf2 Zielgenen in verschiedenen Abschnitten des Darms von Ferkeln zwischen dem Absetzen und Tag 21 nach dem Absetzen

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Weaning of pigs is commonly associated with enteric infections, intestinal dysfunction and villus atrophy. These conditions are accompanied with a depression of feed consumption and animal growth. Development of gut disorders is induced by the activation of inflammatory processes and changes in the expression of inflammatory cytokines. Inflammation is mainly triggered by an activation of the transcription factor nuclear factor kappa B (NF-κB) which controls the expression of many pro-inflammatory genes, including cytokines. Nuclear factor E2-related factor 2 (Nrf2), a redox-sensitive transcription factor, which controls the transcription of various anti-oxidative and cytoprotective proteins and acts as a counterpart of NF-κB. While the occurrence of pro-inflammatory conditions in the intestine in weaned pigs is well known, the time profile of the expression of pro-inflammatory genes in the intestine after weaning has not yet been determined. Therefore, the aim of this study was to investigate the gene expression of pro-inflammatory and anti-oxidative genes in different parts of the intestine at various time points post-weaning.

Methods: 48 crossbred (Danzucht x Pietrain) pigs born from 4 sows, with a birth weight of 1.39 ± 0.201 (SD) kg were used for this experiment. Until day 21, pigs were nursed by their mothers; from day 6, a creep feed was additionally supplied for ad libitum intake. On day 21 all pigs were weaned. At the day of weaning (day 0) and days 3, 7 and 21 post-weaning, 12 pigs (3 from each sow) were killed and mucosa from duodenum, jejunum, ileum and colon ascendens were obtained. In the mucosa samples, relative mRNA abundances of NF-κB and Nrf2 target genes were determined by real-time PCR. Villus heights and crypt depths in duodenum and jejunum were determined by light microscopy at 100x magnification on day 0 and 7. Statistical analysis was done by one-way ANOVA.

Results: In duodenum and jejunum, relative mRNA abundances of pro-inflammatory cytokines, such as interleukin 1 beta (IL1B), interleukin 8 (IL8) and tumor necrosis factor-alpha (TNFa), were increased from day 0 to day 21 post-weaning in duodenum and jejunum ($P < 0.05$). In ileum, IL8 was up-regulated and TNFa was down-regulated ($P < 0.05$), whereas in colon, all NF-κB target genes considered were down-regulated from day 0 to day 21 post-weaning ($P < 0.05$). The expression levels of Nrf2 target genes such as glutathione peroxidase 2 (GPX2), peroxiredoxin 6 (PRDX6) and superoxide dismutase 1 (SOD1) showed the same expression profiles as the NF-κB target genes. In duodenum, GPX2, SOD1 ($P < 0.05$) and PRDX6 ($P < 0.1$) were up-regulated from day 0 to day 21 post-weaning. In jejunum, GPX2 and PRDX6 were up-regulated, whereas in ileum GPX2 was up-regulated and SOD1 was down-regulated ($P < 0.05$). In difference to the small intestinal parts, all of Nrf2 target genes considered in the colon were down-regulated from day 0 to day 21 post-weaning ($P < 0.05$). Heights of villi in duodenum ($P < 0.1$) and jejunum ($P < 0.05$) decreased from day 0 to day 7, while crypt depths and the villus height:crypt depth ratios did not differ between day 0 and day 7.

Conclusion: The present study shows that inflammatory processes in duodenum and jejunum, the main sites of absorption of nutrients, are enhanced from the day of weaning until day 21 post-weaning. This suggests that dietary strategies to counteract the pro-inflammatory condition in the intestine are not only useful in the early weaning period but might be even more effective in the later period of weaning. The finding that the expression of anti-oxidative and cytoprotective Nrf2 target genes paralleled the expression of pro-inflammatory genes might be regarded as compensatory means to counteract the pro-inflammatory condition.

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55. Effects of type and concentration of dietary fat on alkane recovery in broilers

Effekte von Fettart und –menge auf die Alkan-Wiederfindung bei Broilern

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The use of natural alkanes as marker is regarded critical when the feed contains insufficient quantities. Than the diets need to be labelled. For digestibility trials an almost complete recovery of the marker is required, but at least knowledge of the recovery rate and essential influences on that. For alkanes it is known that they might be absorbed (1). Fats may enhance absorption due to the fat-soluble nature of alkanes. The aim was to investigate if natural alkanes are decline in the gastrointestinal tract of broilers in the presence of different quantities of various dietary fats.

Methods: A balance trial with soybean oil, rapeseed oil and palm kernel oil (20, 50 and 80 g/kg dry matter [DM] each), was carried out with Ross-broilers (8/group). In the beginning, the broilers were 12 d old and received 60 g/d of their respective diet. Water was provided *ad libitum*. TiO_2 (5 g/kg DM) was used as inert standard for comparison with natural alkanes C29, C31 and C33 from candelilla wax (0.4 g/kg DM). After 4 days adaptation, excreta were collected quantitatively twice a day during 5 consecutive days. Subsequently, chyme samples were collected in 3 subsections of the small intestine and TiO_2 and *n*-alkanes analyzed in feed, chyme and excreta (2). Excreta marker recovery (EMR) was calculated for *n*-alkanes, and also ratios of TiO_2 to the individual alkanes for chyme and excreta. Analysis of variance and Dunnett-test were performed (SAS 9.3).

Results: For all alkanes, there were no differences in EMR between the nine diets ($P > 0.05$; tab.). Ratio of TiO_2 to alkanes did not differ between gut sections, but between chyme and excreta ($P < 0.05$).

Conclusions: Results indicate that fat type and concentration do not influence EMR of natural alkanes from wax, but there seem to be any kind of alkane loss within the gut. The underlying mechanism remains unclear. It needs further to be clarified whether i) the passage dynamics of alkanes and TiO_2 are congruent or not, and ii) how representative n-alkanes from waxes are for plantal cuticular *n*-alkanes which are somewhat differently embedded in plantal structures.

EMR[3]	Feed variant (Fat of different types, in g/kg DM)									TiO_2/alkanes	
	soybean oil			rapeseed oil			palm kernel oil				
	20	50	80	20	50	80	20	50	80	CH[1]	EX[2]
C29	0.78^{a}	0.79^{a}	0.64^{a}	0.61^{a}	0.52^{a}	0.55^{a}	0.57^{a}	0.66^{a}	0.63^{a}	0.36^{a}	0.40^{b}
	(0.33)	(0.19)	(0.08)	(0.21)	(0.12)	(0.12)	(0.09)	(0.13)	(0.17)	(0.01)	(0.05)
C31	0.84^{b}	0.82^{b}	0.78^{b}	0.85^{b}	0.77^{b}	0.97^{b}	0.84^{b}	0.84^{b}	0.82^{b}	0.05^{a}	0.06^{b}
	(0.35)	(0.13)	(0.10)	(0.23)	(0.08)	(0.14)	(0.07)	(0.13)	(0.18)	(0.00)	(0.00)
C33	0.73^{c}	0.74^{cb}	0.75^{cb}	0.82^{cb}	0.74^{cb}	0.88^{cb}	0.84^{cb}	0.86^{cb}	0.81^{cb}	0.34^{a}	0.38^{b}
	(0.30)	(0.10)	(0.09)	(0.18)	(0.06)	(0.09)	(0.08)	(0.12)	(0.17)	(0.02)	(0.02)
TiO_2	0.97^{de}	0.94^{de}	0.83^{ed}	1.03^{d}	0.97^{de}	1.00^{de}	1.10^{d}	1.04^{d}	0.99^{de}		
	(0.06)	(0.11)	(0.10)	(0.18)	(0.05)	(0.10)	(0.15)	(0.13)	(0.16)		

^{abcd} $P < 0.05$ within treat; [1]chyme, [2]excreta, [3]excreta marker recovery

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56. Comparison of the effect of two phytases alone and in combination with formic acid on phosphorus-, calcium- and zinc retention in pig

Vergleich der Wirkung von zwei Phytasen mit und ohne Ameisensäure auf die Phosphor-, Calcium- und Zinkretention beim Schwein

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Despite the use of phytase in swine nutrition the degradation of phytate in the digestive tract of pigs is incomplete and there is potential to increase the benefits of phytase application. Approaches for improvement in phosphorus (P) release by phytase are the development of more effective and stable phytase molecules. As shown earlier (1) another method to enhance the release of P from phytase containing diets is acidifying such diets with formic acid (FA).The current study was conducted to compare the efficacy of a fungal 3-phytase (PHY3) and a bacterial 6- phytase (PHY6) in pigs. The investigation aimed further to evaluate effects of diet acidification with FA on efficiency of both phytases with regard to P, calcium (Ca) and zinc (Zn) utilization.

Methods: For the experiment 12 barrows ([DExDL] x db77), were assigned to a triplicate 4x4 Latin square design in which 4 dietary treatments were tested in 4 balance periods. Diets were based on wheat, barley and soybean meal and were designed to be marginal deficient in P and Zn (3.7 g/kg, 40 mg/kg, as fed). Diets were: 1) PHY3: basal diet supplemented (suppl.) with 500 phytase units (FTU) PHY3 (Natuphos®, BASF SE, Germany), 2) PHY3+FA diet PHY3 suppl. additional with FA (Amasil® 85, BASF SE, Germany), 3) PHY6: basal diet suppl. with 500 FTU PHY6 (phytase preparation, BASF SE, Germany), 4) PHY6+FA: diet PHY6 suppl. additional with FA. Dosage of FA was 3.3 g/kg as fed. During the trial pigs were housed individually in metabolic cages with free access to water. Artificial lightning was for 14 h per day, room temperature was kept at 22 °C and humidity was in average 70%. Pigs were fed twice daily with pelleted diets. Feed allowance per day was 1060, 1180, 1320 and 1440 g in period I-IV. Each period consisted of 5 days for diet adaptation followed subsequent of 5 days for quantitatively collection of urine and faeces. Data were recorded in a weight range of pigs between 33 and 55 kg. Content of P, Zn and Ca were analyzed in feed, faeces and urine. Apparent total tract digestibilities and retentions were calculated. Statistical analysis was carried out by using the GLM procedure of SAS. The model included animal, period and diet as fixed effects. Results are defined as significant if $p \leq 0.05$.

Results: Results indicate that PHY6 is more efficient in P release than PHY3. Combination of PHY6 with FA had no additional effect on its efficacy. Application of FA in combination with PHY3 is equal to the effect of the single use of PHY6. Parameters for Zn were not significant influenced by the treatments. Zn-digestibility tended to be similar to the results of P. Ca-digestibility was highest for the diet FA+PHY3. Ca-digestibility followed a similar pattern as P regarding to digestibility.

	PHY3	PHY3+FA	PHY6	PHY6+FA	SEM	treatment
P-digestibility [%]	$52.0^{a} \pm 6,9$	$60.1^{b} \pm 4.9$	$58.3^{b} \pm 5.8$	$57.7^{b} \pm 8.2$	1.1	<0.0001
P-Retention [g/d]	$2.3^{a} \pm 0.2$	$2.7^{b} \pm 0.5$	$2.7^{b} \pm 0.5$	$2.6^{b} \pm 0.4$	0.1	<0.0001
Zn-digestibility [%]	24.8 ± 9.3	29.5 ± 9.0	29.1 ± 8.8	29.3 ± 12.4	1.4	0.0793
Zn-Retention [mg/d]	7.5 ± 7.9	11.7 ± 6.8	7.7 ± 7.9	-2.6 ± 31.3	4.3	0.1306
Ca-digestibility [%]	$65.6^{a} \pm 4.9$	$70,0^{b} \pm 5.0$	$66.9^{a,b} \pm 5.9$	$67.5^{a,b} \pm 5.8$	0.8	0.0055
Ca-Retention [g/d]	4.7 ± 1.7	5.3 ± 1.2	5.2 ± 1.2	5.1 ± 1.0	0.3	0.5230
data are means ± standard deviation; n =12 per diet; different letters in a row label significant differences (p< 0.05)						

Conclusions: The effects of PHY3 either alone or in combination with FA are in accordance to earlier findings (1). Combination of FA with PHY3 in diets for growing pigs can replace more mineral P as the supplementation of PHY3 alone. PHY6 showed no improvements in P release through acidification but enabled the same level of P as PHY3 in combination with FA.

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57. Supplementation with Quaternary Benzo(c)phenanthridine Alkaloids Decreases Salivary Cortisol and Salmonella Shedding in Pigs after Transportation to the Slaughterhouse

Futterergänzung mit quarternären Benzophenanthridin-Alkaloiden senkt den Cortisolgehalt im Speichel und das Ausscheiden von Salmonellen bei Schweinen nach dem Transport zum Schlachthaus

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Salmonellosis is one of the leading foodborne diseases worldwide. Several factors have been identified to increase *Salmonella* shedding in pigs and the risk of carcass contamination, including transportation stress. Herbal extracts containing quaternary benzo(c)phenanthridine alkaloids (QBA) have shown a wide range of physiological effects such as anti-inflammatory, antimicrobial and immune-modulatory properties. The study was aimed to evaluate the effect of QBA (Sangrovit®, Phytobiotics GmbH, Eltville, Germany) supplementation on salivary cortisol and *Salmonella* shedding of pigs after transportation to the slaughterhouse. We hypothesized that QBA supplementation would decrease *Salmonella* shedding by regulating stress response.

Methods: A total of 82 pigs (initial body weight 47.9 ± 7.2 kg) were orally challenged with a cocktail of *Salmonella* serovars and randomly assigned to 3 treatment groups (day 14; Treatment 1: T1, in-feed QBA for two weeks; Treatment 2: T2, in-feed QBA for two weeks and water soluble QBA for the last three days; Control: CON, non-supplemented). Pigs were transported to the slaughterhouse after two weeks. Saliva and fecal samples were collected from each individual pig at specific times during the study period, and carcass swabs were obtained after evisceration. Salivary cortisol was measured and fecal samples and carcass swabs were analyzed for detection and quantification of *Salmonella*.

Results: A very high to a moderate positive correlation was found between salivary cortisol and *Salmonella* shedding after transportation to the slaughterhouse in all groups ($P < 0.05$). Overall, mean salivary cortisol decreased in all groups overtime ($P < 0.0001$). However, after transportation CON group showed a significant increase in salivary cortisol ($P < 0.0001$) and the concentrations in CON were higher as compared to T1 ($P = 0.0002$) and T2 ($P < 0.0001$). *Salmonella* prevalence decreased significantly after transportation in T2 ($P = 0.05$) and it was higher in CON group as compared to T1 ($P = 0.02$) and T2 ($P = 0.02$). After transportation CON group showed a significant increase in the number of *Salmonella* shed through the feces ($P = 0.04$), whereas T2 showed a significant decrease in *Salmonella* shedding ($P = 0.009$) as compared to pre-transport levels. Additionally, T2 shed lower amounts of *Salmonella* after transportation to the slaughterhouse than T1 ($P = 0.02$) and CON group ($P = 0.08$). The proportion of *Salmonella*-contaminated carcasses was not different between groups ($P > 0.05$). However, the quantity of *Salmonella* contaminating the carcasses was higher in CON than in T1 and T2 groups ($P = 0.01$).

Conclusion: This study showed that transportation is a stressful event for pigs resulting in increased *Salmonella* shedding in conventionally produced pigs. Additionally, regulating stress response due to transportation by adding QBA+PA to the feed and the water of finishing pigs was effective in reducing the proportion of *Salmonella*-positive pigs as well as the numbers of *Salmonella* shed through their feces, which may contaminate the carcasses. QBA+PA supplementation may be an effective strategy to reduce stress response in pigs and ameliorate its effects on welfare and the quality and safety of the food products.

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58. DON biomarker study in pigs: Efficacy of Biomin® BBSH 797 to biotransform DON to less toxic DOM-1

DON Biomarker Studie in Schweinen: Einsatz von Biomin® BBSH 797 zur effizienten Biotransformation von DON zu dem nicht toxischen Metaboliten DOM-1

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In farm animals, contamination of cereals and related feed products with *Fusarium* toxins causes feed-borne intoxication, especially pigs are very sensitive to such substances. Therefore, efficient analytical tools for qualitative analysis and a regular screening of feed and commodities is required. Within the annual BIOMIN mycotoxin survey, in 2014 (Jan to Oct) around 1,440 samples sourced in Europe were analyzed for aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). Samples were analyzed using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS, Spectrum 380®), high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). The latter method was only used for single commodities.

Results showed a high prevalence of DON (62%), FUM (48%) and ZEN (46%) with average contamination levels of 610 ppb, 920 ppb and 53 ppb, respectively. All of these account for a potential health risk to livestock animals and maximum concentration levels were extremely high with 14,648 ppb DON and 16,024 ppb FUM.

These data highlight the importance of mycotoxin deactivating products to support farm animals, especially in case of the most often occurring mycotoxin DON. Biomarker studies are requested by authorities like EFSA in order to demonstrate the efficacy of mycotoxin deactivating products *in vivo.*

The aim of the present study was to prove the capability of Biomin® BBSH 797 (Genus novus of family *Coriobacteriaceae*; a live bacterium isolated from rumen) to detoxify DON to the less toxic metabolite de-epoxy-deoxynivalenol (DOM-1) in the gastrointestinal tract of pigs by measuring DON and its metabolite DOM-1 in the serum. This study should show the link between mycotoxin exposure to disease risk and also the effectiveness of additives to detoxify mycotoxins to non-toxic metabolites *in vivo*.

Methods: After an adaption period of two weeks, 24 out of 124 weaned piglets (mixed sex, approx. 28 days) were randomly assigned to three experimental groups, according to weight, gender and overall condition. The control group received feed without DON or BBSH 797. The second group received 2 µg/kg of naturally DON-contaminated wheat and the third group received feed containing 2 µg/kg DON and 1.7 x 10^8 cfu BBSH 797/kg. During the experimental phase, piglets were fed restrictively twice a day. Serum samples of all animals in all groups were taken on four consecutive days. Sample 1 (blank serum sample) was taken before feeding the experimental diets. All other serum samples were taken 1.5, 4, 10 and 24 hours after feeding the experimental diets. Serum samples were analyzed for DON and DOM-1 concentrations by LC/MS-MS. All data generated out of the trial were subjected to statistical analysis by the means of ANOVA (SPPS 19.0).

Results: There were no significant differences in blank serum samples (ng/mL±SEM) between the three groups (4.64±0.266; 4.52±0.42; 4.99±0.70). On day three of the trial, DON concentrations 1.5 hours after feeding were more than four times higher in the serum of the DON-group (22.68±4.22) compared to the control (4.73±0.20) and the DON+BBSH group (5.99±0.21) ($P<0.05$). DOM-1 concentrations in the serum (day 3, 1.5 hours) were highest in the DON+BBSH group (15.76±1.58) and differed significantly ($P<0.05$) from the control (traces) as well as the DON-group (1.59±0.25).

Conclusion: To conclude, DON and its metabolite DOM-1 in serum can be used as biomarkers under experimental conditions to test products for their mycotoxin degrading activity *in vivo*. Results of this study revealed a significant reduction of the DON concentration in the serum of animals receiving Biomin® BBSH 797.

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59. Impact of zinc oxide on the development of enterobacterial antibiotic resistance genes directly after weaning in piglets

Einfluss von Zinkoxid auf die Entwicklung enterobakterieller Resistenzgene gegen Antibiotika unmittelbar nach dem Absetzen in Ferkeln

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Question: Dietary zinc oxide in high doses (up to 3g/kg feed) is used in many countries in piglet nutrition to successfully combat *E. coli* induced post weaning diarrhea in piglets. However, the antibiotic effect of dietary ZnO also leads to a drastically modified intestinal microbiota of freshly weaned piglets with possible detrimental effects later in life. Furthermore, there are indications that ZnO increases the occurrence of multi-resistant *Escherichia coli* strains and also increases the occurrence of enterobacterial tetracycline and sulfonamide genes in the intestinal tract of weaned piglets. As the time frame of the development is still unclear, the aim of this study was to closely monitor the development of enterobacterial antibiotic resistance genes in piglets directly after weaning.

Material and methods: Weaned piglets (n=6 per group) were either fed diets with medium (150 mg/kg) or high (3000 mg/kg) zinc from analytical grade zinc oxide and 150 or 300 mg/kg zinc from a commercial ZnO preparation. Fecal samples were taken at 0,1,2,3,4,6,8,10 and 14 days and subsequenct DNA extracts were amplified by qPCR assays to quantify copy numbers for the enterobacterial tetracycline (*tet*A), sulfonamide (*sul*1) and quinolone (qnrA) resistance gene, respectively. Additionally, data on 16S rRNA gene copy numbers from the *Escherichia* group were used to estimate the impact of dietary zinc oxide on the development of enterobacterial antibiotic resistance. Data was analysed by the Kruskal-Wallis test, followed by Mann-Whitney U test, where appropriate.

Results: During the first two days after weaning, fecal copy numbers of *Escherichia* in the high ZnO group surprisingly increased compared to day 0, while the diets with medium ZnO and the commercial ZnO preparation showed higher concentrations on day two and three, respectively. Beginning on day three and four, a sharp decline of fecal *Escherichia* copy numbers was apparent in all groups, being most pronounced in animals fed the high ZnO and the 300 mg ZnO/kg diet, respectively.

However, while the medium ZnO and the 300 mg ZnO/kg diets only led to slight increases for the *tet*A resistance gene after six days, animals fed the high ZnO diet showed drastically increasing *tet*A copy numbers, which led to a significantly higher amount on day 10 and 14. The *sul*1 resistance gene showed the same but delayed trend after eight days. The amount of *qnr*A copy numbers was much lower than for *tet*A and *sul*1, respectively. However, in piglets fed the high ZnO diet, a sharp increase was noticed for the *qnr*A quinolone resistance gene already six days after weaning. The comparison of enterobacterial 16S rRNA gene- and resistance genes copy numbers showed that the relative amount of antibiotic resistant *Escherichia* spp. increased.

Conclusions: A zinc oxide induced reduction of *Escherichia* spp. started three to four days after weaning, but increased antibiotic resistance especially against tetracycline and sulfonamides developed in the second week in animals fed high doses of dietary zinc oxide. Thus, it is concluded that the main effect of high doses of dietary ZnO takes place during the first week after weaning. A further application of dietary zinc oxide may however increase the shedding of antibiotic resistant *Escherichia* spp. into the environment.

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60. The effect of different application forms of (n-)butyrate on the intestinal activity of cytochrome P450 enzymes in chicken

Die Wirkung verschiedener Formen von (n-)Butyrat auf die intestinale Cytochrom P450 Enzymaktivität beim Huhn

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The short chain fatty acid (n-)butyrate is commonly used as a natural growth promoter in poultry nutrition in various application forms. Due to its receptor-mediated and epigenetic effects, orally administered butyrate may alter the expression of various genes, such as those of hepatic microsomal cytochrome P450 (CYP) enzymes, highly involved in detoxification and drug metabolism. Although, modification of hepatic CYP gene expression was found after oral butyrate administration in chicken, finally it has not been realized on the level of enzyme activity in the liver. Since CYP enzymes are present in the small intestine as well and are responsible for the majority of intestinal phase I drug metabolism reactions, the possible influence of dietary factors on intestinal CYP enzyme activity could have high practical significance in poultry nutrition. The present study aimed to investigate the changes in intestinal activity of CYP enzymes following application of sodium salt of n-butyric acid (non-protected sodium butyrate) and micro encapsulated form of butyrate (protected butyrate - ButiPEARL) as feed additives and feeding a diet with high non-starch polysaccharide (NSP) content, which serves as a precursor of endogenous butyrate production in the large intestine. We also studied the distribution of butyrate in various parts of the intestinal tract and measured the plasma butyrate concentration after different application forms of butyrate.

Methods: One-day old Ross 308 broiler chicken (n=22/group, 172 in totalwere fed a maize-based (MB) or wheat-based diet. To enhance NSP digestibility, carbohydrase was added to the wheat-based diet (WBES). Beside MB and WBES control diets, experimental diets contain either two different dosages (1.5 or 3.0 g/kg diet) of sodium butyrate or protected butyrate (0.2 g/kg diet);all diets were fed from day 1 to 42.,. After 42 days animals were anesthetized for blood sampling (n=6/group), and were slaughtered for intestinal content (n=10/group) and intestinal mucosa sampling (n=6/group) in order to determine CYP enzyme activity. Butyrate concentration of the intestinal content from duodenum, ileum and caecum, further, plasma butyrate concentration from the hepatic portal veins (*Vena gastropancreaticoduodenalis* and *Vena mesenterica communis*) and systemic circulation (*Vena brachialis*) were determined by gas chromatography. Intestinal CYP1, CYP2 and CYP3 enzyme activity of duodenal epithelial cell (enterocyte) microsomes were measured by luminescent CYP Glo Assays. Data were analyzed by two-way ANOVA and pairwise comparison using the R 2.14.0 software.

Results: Dietary sodium butyrate supplementation did not have any significant effect on the butyrate concentration in duodenum, ileum and caecum either with basal or with elevated dose, which indicated that sodium butyrate was absorbed from the gut lumen before reaching the small intestine. In contrast, protected butyrate supplementation increased the butyrate concentration in the ileum ($p<0.01$), while WBES diet resulted in elevated butyrate concentration in the caecum ($p<0.01$). Plasma butyrate concentration in *Vena brachialis* was affected by elevated sodium butyrate supplementation only ($p<0.001$). In *Vena gastropancreaticoduodenalis* the elevated dose of sodium butyrate supplementation and the WBES diet resulted in higher plasma butyrate concentration ($p<0.001$, $p<0.1$ respectively), however, in *Vena mesenterica communis* all elevated dose of sodium butyrate supplementation, protected butyrate supplementation and WBES diet increased plasma butyrate concentration ($p<0.001$). CYP1 and CYP2 activity in duodenal epithelial cells were increased by both elevated dose of sodium butyrate supplementation and WBES diet (CYP1: $p<0.01$, $p<0.001$; CYP2: $p<0.01$, $p<0.05$ respectively), while CYP3 activity was influenced by the diet type only ($p<0.001$).

Conclusion: Our present study proved, that both dietary and endogenous butyrate produced in the large intestine are able to alter the activity of CYP enzymes in the duodenal epithelial cells. These results may suggest that the induction of intestinal CYP enzymes could origin not only from the intestinal luminal butyrate content but probably also from basolateral butyrate of portal and systemic circulation as well. Such action of butyrate can be of special importance from food safety and pharmacotherapeutical point of view, as it can modify the metabolism and intestinal kinetics of simultaneously applied drugs and xenobiotics.

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61. Bioavailability of quercetin from different onion extracts in cattle

Bioverfügbarkeit von Flavonoiden aus verschiedenen Zwiebelextrakten bei Rindern

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The secondary plant metabolite quercetin is one of the most abundant and investigated flavonoids with health-promoting properties. Recent studies in cows have shown a higher bioavailability of quercetin from its glucorhamnoside rutin than of the aglycone after intraruminal (i.r.) application. To further investigate the impact of the sugar moiety we determined the bioavailability of quercetin from two onion extracts as a natural source of quercetin agylcone and various quercetin glycosides.

Material and Methods: The study was performed in 3 rumen-fistulated non-lactating cows (Deutsches Schwarzbuntes Niederungsrind, mean bodyweight (BW) 554 ± 9 kg (mean ± SEM)) equipped with indwelling catheters placed in one jugular vein. The animals were fed a diet consisting of 1.5 kg concentrate and 1.5 kg hay (TS) twice daily. After i.r. application of equimolar amounts of quercetin [50mg/kg of BW] either as extracts of onion bulb (OBE, 50% aglycone and 50% glycosides) or onion skin (OSE, almost 100% aglycone), as rutin (R, 100 % glycoside) or as quercetin aglycone (Q, 100 % agylcone) with the morning feeding, blood samples were drawn at various time intervals (0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24h after application). The plasma concentration of quercetin and its metabolites with an intact flavonol structure (kaempferol, isorhamnetin, tamarixetin) were analyzed by HPLC with fluorescence detection. Bioavailability of quercetin from the different sources used was judged by calculating the area under the plasma concentration curve (AUC) as well as c_{max} (maximal plasma concentration) and t_{max} (time point of c_{max}). The results were statistically evaluated by one way ANOVA with pair-wise comparisons of group means according to the post hoc tukey-kramer test ($P < 0.05$) using SAS (SAS Institute, Inc., Version 9.2).

Results: With the exception of t_{max}, the pharmacokinetic parameters differed between treatment groups (Tab). Compared with Q and OSE, the AUC for total plasma flavonols (sum of conjugated and non-conjugated quercetin and its derivatives) after application of R and OBE were significantly larger. In principal c_{max} followed the significance pattern of AUC with the exception that groups R and OBE were additionally different.

Tab.: Pharmacokinetic parameters after i.r. application of 50 mg/kg BW quercetin equivalents from rutin (R), quercetin (Q), onion skin extract (OSE) onion bulb extract (OBE)

	R	Q	OSE	OBE
c_{max}	950.8 ± 255.5^{a}	109.7 ± 42.9^{b}	96.7 ± 10.6^{b}	1647.7 ± 195.9^{c}
t_{max}	1.0 ± 0.0^{a}	3.2 ± 2.4^{a}	0.8 ± 0.3^{a}	0.7 ± 0.2^{a}
AUC_{0-24}	1547.5 ± 495.5^{a}	379.1 ± 84.8^{b}	265.9 ± 53.8^{b}	2349.3 ± 343.5^{a}

Means ± SEM, n = 3; means within a row bearing no common superscript differ significantly ($P \leq 0.05$); c_{max} [nmol/l] = maximal concentration, t_{max} [h] = time point of maximal concentration, AUC [nmol/l × min] = area under the curve of total flavonols

Conclusion: Although there was no significant difference between R and OBE concerning the AUC one must take into account that on a molar basis OBE contains only 50% of its total quercetin as glycosides whereas R consisted of 100% glycosides. Thus, it can be speculated that the application of 100% of the dose as OBE glycosides would result in even higher bioavailability of quercetin from this source compared with R.

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62. Effects of 6-wk intraduodenal supplementation of quercetin on blood parameters and energy metabolism in periparturient dairy cows

Effekte einer 6-wöchigen intraduodenalen Quercetinsupplementation auf Plasmaparameter und Energiestoffwechsel bei der peripartalen Milchkuh

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The periparturient period poses a time period of metabolic challenges for dairy cows resulting in negative energy balance (EB) and a range of health problems post partum (p.p.). To compensate negative EB, cows mobilize fatty acids from adipose tissue which can lead to fatty liver (1). Flavonoids, such as quercetin (Q), are polyphenolic substances found in all higher plants, have hepatoprotective potential and the ability to prevent or reduce hepatic lipid accumulation (2). In ruminants, Q is largely degraded in the rumen, and knowledge on metabolic effects of Q is scarce. To explore Q post-ruminal absorption and metabolism we administered it directly in the duodenum (3). In this study we investigated whether intraduodenally administered Q acts beneficially on EB and parameters of lipid metabolism and has hepatoprotective effects in periparturient dairy cows.

Methods High yielding German Holstein dairy cows (n=10), each equipped with a duodenal fistula, were fed grass silage ante partum (a.p.) and a TMR ad libitum p.p. and monitored from 4 weeks (wk) a.p. to 3 wk p.p.. Starting at 3 wk a.p., in 5 cows 100 mg Q dihydrate/kg BW were administered daily in the duodenum for 6 wk while control cows received sodium chloride solution. Cows were transferred to respiration chambers 1 wk before and after 1 and 5 wk of treatment. Production of CO_2 and CH_4, O_2 consumption and feed intake were measured. Energy expenditure, fat and carbohydrate oxidation, and EB were calculated individually. Blood samples were drawn 4 times/wk for analysis of plasma flavonoid content and parameters (non esterified fatty acids, beta-hydroxybutyric acid, triglycerides, cholesterol, glucose, albumin, urea, aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH)) related to energy metabolism and liver health.

Results Intraduodenal Q supplementation resulted in higher plasma flavonoid levels than in control cows (167 ± 23 vs. 5 ± 24 nmol/L; $P<0.05$) and in a tendency for higher plasma flavonoid values ($P=0.09$) in Q cows p.p. compared to a.p. values, possibly due to reduced Q metabolism associated to metabolic stress in early lactation. Q cows did not show differences in metabolic status and EB compared to control cows, but the increase in plasma AST activity p.p. was 80% less in the Q treated group than in control cows ($P<0.05$). Additionally, the increase of plasma GLDH in the Q group was also lower, but not significant ($P=0.15$). There were no Q effects on energy expenditure, but cows showed a decline in respiratory quotient ($P<0.05$) from a.p. to p.p., and fat oxidation peaked after calving, indicating the higher energy supply from fatty acids derived from lipomobilization.

Conclusions Q supplementation might be beneficial in periparturient cows because plasma AST and GLDH activities as parameters of liver damage were reduced.

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63. Influence of a grape seed and grape marc meal extract (GSGME) on milk yield, hepatic expression of genes involved in inflammation and endoplasmic stress response and antioxidant parameters in dairy cows

Einfluss von Traubentrester auf Milchleistung, die Expression von Genen der Entzündung und der Antwort auf Stress des endoplasmatischen Retikulums in der Leber sowie den antioxidativen Status bei Milchkühen

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The transition period represents the most critical period in the productive life of high yielding dairy cows. Over the first weeks of lactation all cows develop a negative energy balance which leads to metabolic stress with undesirable effects on liver metabolism. Therefore the production of cytokines in the liver is enhanced, which in turn leads to the occurrence of inflammation and endoplasmic reticulum (ER) stress which causes an impairment of hepatic function and the health status of high yielding dairy cows. By-products of wine/grape juice production are characterized by a high content of polyphenols. Studies in several animal models have shown that polyphenols have strong anti-inflammatory properties. The present study investigated the hypothesis that feeding a polyphenol-rich grape seed and grape marc extract (GSGME) has the potential to suppress the inflammatory process and ER stress in the liver of dairy cows during early lactation.

Methods: Twenty eight primi- and multiparous dairy cows (German Holstein) were divided into a control group (n=14) and a group receiving GSGME from wk 3 ante partum (a.p.) to wk 9 post partum (p.p.) (n=14). The average number of lactation in these groups was 2.8 ± 1.6 and 2.9 ± 1.5, mean ± SD, respectively. All cows received a total mixed ration (TMR). The GSGME group received additionally a GSGME (with a polyphenol content of 43 mg/g; Antaox®, Dr. Eckel Niederzissen) as a component of TMR (1 % of DM). Milk samples were taken weekly from wk 1 to wk 9 p.p. Blood samples were taken in wks 1, 3 and 5 from *vena caudalis mediana* after the morning milking before feeding. Liver biopsies were taken from the right liver lope at wks 1 and 3 p.p. mRNA concentrations of hepatic genes were determined by qPCR. Data were statistically evaluated by the Linear Mixed-Effects Model of R (version 3.1.1). The model included treatment, week, lactation number and the treatment x week interaction as fixed factors and cow as random factor.

Results: Dry matter intake of the cows did not differ between the two groups of cows. However, the GSGME group had a greater milk performance (milk yield: + 3.7 kg/d; p=0.022; energy corrected milk: + 2.9 kg, p=0.049) from wks 2 to 9 of lactation. Cows of the GSGME group had lower mRNA concentrations of FGF21, a key marker of ER stress, in the liver (-67%; p=0.014). In this group, there was also a reduction of the mRNA concentration of various other genes involved in ER stress response such as ATF4 (-20%), BAK1 (-21%), BAX (-19%), DDIT3 (-26%), EDEM1 (-16%), BIP (-45%) and XBP1 (-51%) and of various pro-inflammatory genes such as CRP (-20%), HP (-55%) and TNFα (-28%) compared to the control group. Although the differences in these genes were not significant, the whole expression pattern of these genes suggests that GSGME suppressed ER stress and inflammation in the liver. Plasma NEFA concentration was not different between both groups, while plasma concentration of BHBA was increased in the GSGME group (p=0.004). Concentrations of α-tocopherol, ß-carotene, TBARS and the total antioxidant capacity in plasma did not differ between both groups. Plasma retinol concentration was increased in the GSGME group (P=0.033).

Conclusion: Feeding GSGME from wk 3 a.p. to wk 9 p.p. increased milk yield in dairy cows. Data of gene expression analyses suggest that this effect might be - at least in part - due to a suppression of inflammation and ER stress in the liver during early lactation while feeding GSGME had no general effect on the antioxidative status. In overall, the study suggests that feeding polyphenol-rich plant extracts during early lactation could be a useful strategy to prevent inflammatory processes and ER stress in the liver and thus improve milk yield and animal health in dairy cows.

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64. Effects of monensin and essential oils on the energy metabolism of periparturient dairy cows

Auswirkungen von Monensin und ätherischen Ölen auf den Energiestoffwechsel von Milchkühen in der peripartalen Phase

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The periparturient dairy cow is confronted with massive changes in metabolism that often result in a negative energy balance. The postulated mechanism of monensin and essential oils (EO) is a shift of the ruminal microflora towards gram- negative bacteria accompanied by an increased production of propionate, the main gluconeogenic precursor. The aim of the study was to examine if monensin or EO have an antiketogenic effect.

Methods: Fiftyseven pluriparous German Holstein cows were allocated six weeks ante partum (a.p.) to either a body condition score (BCS) higher 3.88±0.1 or BCS lower 2.81±0.1 (LC) group. LC group (n=14) represents a control group not being in a ketogenic metabolic status. BCS higher group was further split up into one group serving as a negative control group (HC, n=13) and two groups receiving either monensin (HC/Mo, n=14) or essential oils (HC/EO, n=15). LC group was fed an energetically adequate ration with 20% concentrate in the ration. HC group received an energetic oversupply with 60% concentrate. After calving the forage:concentrate ratio was changed stepwise from 70:30 to 50:50. This increase was decelerated (3 vs. 2 weeks) in the HC group to additionally enhance post partal lipolysis. HC/Mo group was administered a monensin-releasing intraruminal bolus (Kexxtone, Elanco) 3 weeks before calving. HC/EO group received a mixture of essential oils (CRINA® Ruminants, DSM) from day 21 a.p. until day 56 postpartum (p.p.). BCS was recorded weekly. Blood samples were taken on day -42, -14, -7, -3, 1, 7, 14, 21, 28, 35, 42, 56 relative to calving for clinical-chemical parameters β-hydroxybuyrate (BHB) and non-esterified fatty acids (NEFA). All statistics were performed by using the MIXED procedure of SAS (Version 9.2) for repeated measures in a compound symmetry covariance structure. The model contained experimental treatment, time and the interaction between the factors. $P < 0.05$ was assumed to indicate significant differences.

Results: Cows of HC group showed a significantly higher BCS ($P \leq 0.01$) than LC group before calving (Fig.1). NEFA of HC group was significantly ($P \leq 0.01$) higher on day 7 than in LC group. NEFA of HC/Mo and HC/EO group were 25 and 17 % lower than in HC group on day 7 p.p. BHB of HC/Mo group was lower than in the HC and HC/EO group and it was significantly ($P \leq 0.01$) lower on day 28 and 35 p.p. compared to HC/EO group (Fig. 2).

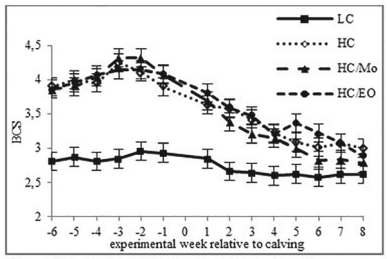

Fig.1 Body condition score (LSMeans±SEM)

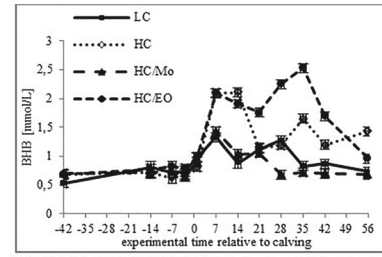

Fig.2 Values of BHB in blood serum (LSMeans±SEM)

Conclusion: BCS, NEFA and BHB of HC and LC cows imply that it was possible to generate animal groups subjected to a ketogenic metabolic status. Animals receiving monensin were less subjected to fat mobilization and ketosis while EO supplementation seems to have no benefit on the energy metabolism under the present conditions.

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65. Tissue distribution of quercetin in rainbow trouts after feeding of quercetin-containing diets over 4 weeks

Gewebeverteilung von Quercetin bei der Regenbogenforelle nach vierwöchiger Applikation mit dem Futter

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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 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. However, for any systemic effects in animals as well as for an accumulation in tissues a sufficiently high bioavailability of quercetin and/or biologically active metabolites is a prerequisite. There is, however, only very limited information about the bioavailability of the flavonol quercetin in fish. Thus the aim of the study was to investigate the effect of chronic feeding of various doses of quercetin on blood and tissue concentrations of quercetin and its metabolites with an intact flavonol structure (kaempferol, isorhamnetin, tamarixetin) in rainbow trouts.

Materials and Methods: The feeding trial was conducted in the experimental facilities of the Gesellschaft für Marine Aquakultur mbH (Büsum, Germany). Dietary treatments consisted of a flavonoid free control diet (30 % fish meal, 27 % wheat flour, 15.5 % soybean-protein-isolate, 15.5 % corn gluten, 7.5 % fish oil) supplemented with 0, 0.83, 2.5, or 4.2, g/kg quercetin aglycone (Roth; Germany). All diets were manufactured by Altromin Spezialfutter GmbH & Co. Kg, Lange, Germany and formulated according to the nutrient requirement recommendations of the National Research Council, USA. Rainbow trouts (Forellenzucht Trostadt GbR, Trostadt Germany) with a mean body weight of 196.7 ± 0.5 g were housed in 16 tanks (150 L, 17 fish per tank) with 4 replications for each dietary treatment. After an acclimatization period of 2 weeks (control diet), fish were fed their respective experimental diets, once a day (16:00 h) for 4 weeks. Diets were fed by hand at a rate of 1.2 g/100 g of body weight (BW), resulting in a final daily dose of 0, 10, 30 and 50 mg/kg BW. On days 0, 1, 3, 7, 14, 21, 28 of the experiment, 2 fishes from each tank were removed and blood plasma, liver and muscle samples were taken and stored at -70 °C until analysis. Blood and tissue samples were analysed for their total flavonol content (quercetin, kaempferol, isorhamnetin) by HPLC with fluorescence detection. Data were statistically analysed according to a two-factorial repeated measure design (dose, time, dose × time) using Proc mixed (SAS). Comparison of means were performed using the Tukey Test.

Results: Tissue concentrations (blood, muscle, liver) of total flavonols significantly increased with the quercetin dose fed (Tab. 1) However, no accumulation of individual as well as of total flavonols over the entire period occurred. Flavonol content in muscle and liver were considerably higher compared to blood plasma levels. The main metabolite in all tissues was quercetin (69-95%), followed by isorhamnetin (4-29 %) and kaempferol (1-3 %).

Tab. 1: Mean tissue concentrations of total flavonols (average over the entire period) in rainbow trouts fed diets with different quercetin content over a period of 4 weeks

	Dose, mg/kg body weight				
	0	10	30	50	SEM
Plasma, nmol/L	0.00^{a}	9.65^{a}	27.94^{b}	42.14^{c}	3.56
Liver, nmol/g	0.24^{a}	0.64^{a}	1.25^{b}	2.32^{c}	0.16
Muscle, nmol/g	0.26^{a}	0.42^{a}	0.76^{a}	1.81^{b}	0.18

Values within a row bearing no common superscript differ significantly ($P < 0.05$).

Conclusion: We conclude from the present results, that the bioavailability of quercetin in rainbow trouts is positively correlated with the quercetin dose applied. However, feeding of quercetin over a period of 4 weeks does not result in time-dependent tissue accumulation of quercetin and its metabolites with an intact flavonol structure.

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66. Effects of low dietary inclusion of red alga Nori on growth and and feed utilization of rainbow trout

Geringe Mengen Rotalgen im Futter erhöhen kurzzeitig das Wachstum von juvenilen Regenbogenforellen

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Red alga Nori can be grown in integrated multi-trophic aquaculture systems, improving the environmental impact of salmon farming. Red algae of the genus *Porphyra* are usually protein rich and contain 30-45% protein. They have been tested with varying success as fishmeal replacements in diets for, among others, rainbow trout (*Oncorhynchus mykiss*). Furthermore they contain around 30% porphyran, a sulphated polysaccharide with immunostimulating properties. As one frequently reported result of immunostimulation is an improvement of growth performance and nutrient utilization, low amounts of Nori have been added to test for their performance.

Methods: Four concentrations (0.625%, 1.25%, 2.5%, 5%) of dried red alga Nori (*Porphyra yezoensis*) were included into rainbow trout diets with a crude protein (CP) content of 500 g/kg DM, a crude lipid (CL) content of 160 g/kg DM, a crude ash content of 100 g/kg DM and a gross energy content of 21.6 kJ/g DM. The same diet without Nori served as control. A total of 750 juvenile (4.11 g ± 0.08, mean ± SD) were stocked at 25 fish each into 30 aquaria. Feed was allocated to 6 replicate aquaria per treatment group. Once a week the fish were weighed and the feed ration adjusted according to the new body mass. They were fed 2.5% of live body mass per day for six days a week and were not fed for 24 hours prior weighing. At the end of the eight week experiment, fish were individually weighed, euthanized with 150 mg/L MS-222, homogenised and proximate composition, feed and nutrient utilizations determined. Data were analysed with SPSS 10.0 applying a one-way ANOVA with diet as fixed factor.

Results: At the end of the eight weeks, no difference between control and Nori fed fish was observed in growth or feed utilization. However, at experimental half-time all Nori fed fish had a higher body mass than control fed fish. Improvement in growth showed a dose dependent pattern with a binomial shape and Nori 1.25 fed fish showed the highest growth increase of all groups.

	Control	N 0.625	N 1.25	N 2.5	N 5	p-value
Initial body mass [g]	4.05	4.05	4.15	4.15	4.15	> 0.05
Body mass 4 weeks [g]	7.58[a]	7.96[b]	8.13[b]	7.96[b]	7.91[b]	< 0.001
Final body mass [kg]	14.4	14.5	14.5	15.0	14.8	> 0.05
Feed conversion ratio [g feed/g body mass gain]	1.00	1.01	1.01	1.00	1.01	> 0.05
Specific growth rate [%/day]	2.28	2.27	2.28	2.29	2.24	> 0.05
Fish composition (FM)						
Crude protein [g/kg]	15.1	15.2	15.1	15.3	15.1	> 0.05
Crude lipid [g/kg]	9.86	10.2	9.94	9.48	9.64	> 0.05
Crude ash [g/kg]	2.46	2.46	2.43	2.59	2.48	> 0.05
Gross energy [kJ/g]	7.55	7.71	7.57	7.44	7.45	> 0.05

Conclusion: Physiologically it is highly interesting that after four weeks the inclusion of Nori resulted in a dose dependent improvement of growth; however the mechanisms behind this effect remain unclear. Since at the end no significant difference between groups was observed the effect of Nori inclusion seems to wear off and might even lead on a long term basis to reduced growth although this was not tested and will need further investigations.

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67. Bioavailability of a single dose of catechins admininstered intraduodenally in high-yielding German Holstein cows

Systemische Verfügbarkeit von Catechinen nach duodenaler Applikation bei hochleistenden Deutsch-Holstein Kühen

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Catechins are phytogenic substances which belong to the group of flavanols. They occur in tea, grapes, apples and other plants. Usually, there are six kinds of catechins mainly found: (+)-catechin (C), (-)-epicatechin (EC), (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG). They have antioxidative and anti-inflammatory properties, show effects on lipid metabolism, and thus might be potentially beneficial in periparturient cows. To be health-promoting these substances must be systemically available. However, catechins are likely degraded by the rumenal microbiota, and nothing is known about its efficacy in dairy cows. Thus, in a first step, we investigated the bioavailability (BA) of catechins after duodenal administration to gain principle information about its post-ruminal absorption and metabolism.

Methods: Six German-Holstein cows at 83-88 days in milk in their 2nd lactation were surgically equipped with duodenal cannulas (1). After recovery a green tea catechin-enriched product (POLYPHENON 60; Sigma) with EGCG and EGC as the main catechin components (~70% of total catechins) was administered in NaCl solution into the duodenum at 3 different dosages (10, 20, 30 mg/kg BW). In addition, a control study with intraduodenal application of NaCl solution was conducted in each cow. Before and after administration of the test compounds plasma samples were taken for a period of 36 h. Between the runs a wash-out period of 2 days was observed to avoid carry-over effects. Concentrations of plasma C, EC, GC, EGC, ECG, EGCG, and total catechins (TC) were measured by HPLC with electrochemical detection. Statistical evaluation was performed using GLM and PROC MIXED of SAS. Data is presented as means ± standard error.

Results: At the day of the BA study cows weighed on average 602.6 ± 8.5 kg, milk yield was between 36 to 40 kg/d, and dry matter intake 22 to 24 kg/d cows (dosage effect $P > 0.62$). After catechin administration plasma concentrations of EC, EGC, EGCG, and TC clearly increased above baseline values in a dose-dependent manner ($P < 0.0001$). For plasma C, ECG, and GC values did not differ from baseline irrespective of the dose administered ($P > 0.5$). In contrast, plasma conncentrations of EC, EGC, EGCG, and TC peaked ($P < 0.01$) between 1.5 and 2.5 h after administration of 20 and 30 mg/kg BW catechins ($P < 0.05$) and returned to baseline values between 2.5 and 4 h after administration, respectively. In the baseline samples no catechins were detected and after administration of 10 mg/kg BW plasma values were not different from baseline ($P > 0.5$). With a dose of 30 mg/kg BW the maximal plasma concentrations were 94, 123, 146, and 382 nmol/L for EGC, EC, EGCG, and TC, respectively.

Conclusions: Peak time of catechins after duodenal administration in dairy cows were similar to those found after oral administration of EC in humans and rats but maximal plasma concentrations were much lower (2) suggesting a rather low BA similar as observed in rats (3). Plasma EGCG showed the highest concentration which agrees with EGCG being the most prominent catechin in the administered product. Species specific intestinal microbiota as well as first pass effects could be possible explanations for the low BA in ruminants. Once catechin efficacy is proven in cows, a rumen-protected preparation has to be developed.

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68. Effects of prepartum energy supply and nicotinic acid supplementation on immunological and biochemical parameters of periparturient dairy cows differing in parity

Effekte der präpartalen Energieversorgung und eines Nikotinsäurezusatzes auf immunologische und biochemische Parameter von peripartalen Milchkühen unterschiedlicher Paritäten

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To compensate negative energy balance, periparturient cows showed enhanced fat mobilization followed by a higher release of non-esterified fatty acids (NEFA) [1]. Increased NEFA concentrations impair immune function [2]. Feeding a high energy-dense diet to prepartum dairy cows with a high body condition (BCS) is associated with an increased production performance postpartum (p.p.) resulting in a more tensed metabolic situation [1]. Nicotinic acid (NA) is known to down-regulate lipolysis in bovine fat tissue [3] and is suggested to balance catabolic metabolism p.p.. The present study aimed to investigate the effects of prepartum diets varying in energy density and NA supplementation on immunological and biochemical parameters of periparturient dairy cows differing in parity, but not pre-selected for high or low BCS.

Methods: 47 German Holstein cows were allocated homogenously to one of four treatment groups (36 pluriparous and 20 primiparous cows). Mean BCS was 3.4 ± 0.4 at the beginning of the experiment and 3.5 ± 0.4 at calving. Cows received either 0 or 24 g NA and 30% (LC) or 60% (HC) concentrate with the rest being a roughage mixture (50% corn and 50% grass silage on DM basis) prepartum. All feeding groups received a concentrate proportion of 30% after calving which was increased up to 50% within 16 d for LC groups and within 24 d for HC groups. Blood samples for the analysis of immunological and biochemical parameters were taken on d -42, -21, -14, -7, -3, 3, 7, 14, 21, 28, 35, 42, 63, 84 and 100 relative to calving. The cell viability and the mitogen stimulated proliferation of peripheral blood mononuclear cells (PBMC) were evaluated on d -42, -14, 3, 7, 14, 28, 42 and 100 relative to calving by alamar blue assay. Statistical analyses were carried out by PROC MIXED procedure of SAS, including dietary concentrate proportion, supplementation, parity, and period as fixed effects as well as the 4-way interaction of those factors. The cow within diet was conducted as random effect and the sampling time as repeated measure. $P < 0.05$ was assumed to indicate significant differences.

Results:

Feeding prepartum diets high in energy density did not affected investigated parameters of dairy cows during late pregnancy and early lactation. All investigated parameters were influenced by period. Parity-related differences were observed for nearly all investigated parameters. Primiparous cows showed increased numbers of leukocytes ($P = 0.001$), absolute lymphocytes ($P < 0.001$), relative lymphocytes ($P = 0.001$) and absolute granulocytes ($P = 0.050$). Contrary, pluriparous cows showed higher numbers of relative granulocytes ($P = 0.028$), ß-hydroxybutyrate concentrations ($P = 0.028$) and activities of gamma-glutamyl transferase (ɣ-GT) ($P = 0.002$). Parity affected also proliferation of PBMC ex vivo ($P = 0.007$) resulting in a higher proliferation capability in primiparous cows. NA supplementation increased activities of glutamic-oxaloacetic-transaminase ($P = 0.022$) and ɣ-GT ($P = 0.061$) during periparturient period.

Conclusion:

Feeding an energy-dense diet prepartum did not lead to metabolic imbalances or deficits in immune function of dairy cows and they responded different compared to cows pre-selected for a high BCS. The influence of parity on several investigated parameters indicate parity-related differences in metabolic status which may be due to a higher milk performance and, therefore, a more pronounced negative energy balance p.p. of pluriparous cows. NA supplementation was not able to balance catabolic situation p.p., but seemed to have impacts on liver metabolism.

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69. Bioavailability of catechins from a green tea extract after intraruminal application in cattle

Bioverfügbarkeit von Catechinen aus einem Grünteeextrakt nach intraruminaler Applikation beim Rind

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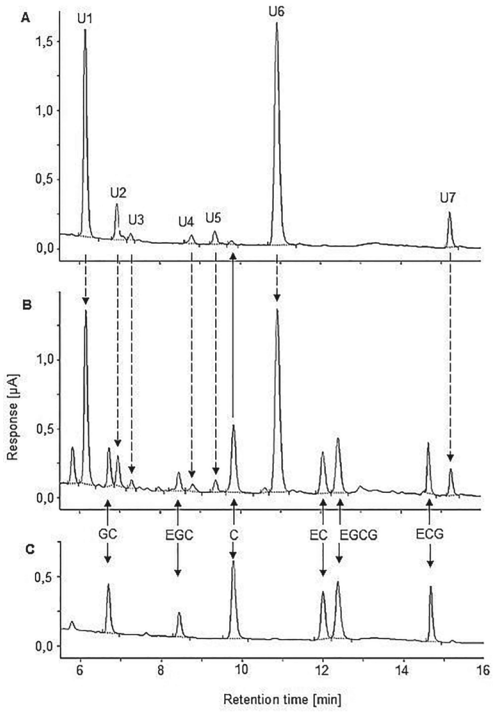
Catechins are a class of flavonoids widely distributed in fruits and beverages including green tea, grapes and apples, which are often components of human diets. Various *in vitro* and *in vivo* studies in humans and rats showed positive effects of green tea extracts, such as antioxidative and anti-inflammatory effects. In particular, high yielding dairy cows with metabolic stress in early lactation could benefit from those effects of catechins. At present, only very limited knowledge about the bioavailability of catechins in cattle is available. Therefore we investigated the bioavailability of catechins after intraruminal (i.r.) application of a green tea extract (Polyphenon 60, Sigma Aldrich; 70 % total catechins). The major catechins in this green tea extract are epigallocatechingallat (ECGC), epigallocatechin (EGC), epicatechingallat (ECG), epicatechin (EC), gallocatechin (GC) and catechin (C).

Methods: The study was performed in 5 rumen-fistulated non-lactating cows (Deutsches Schwarzbuntes Niederungsrind, mean bodyweight (BW) 479 ± 15 kg (SEM)) equipped with indwelling catheters placed in one jugular vein. The animals were fed a diet consisting of 1.5 kg concentrate and 1.5 kg hay (TS) twice daily. After i.r. application of Polyphenon 60 in two different dosages (10 and 50 mg/kg of BW) with the morning feeding, blood samples were drawn at various time intervals. The concentration of the major catechins (GC, EGC, C, EC, EGCG, ECG) in plasma samples were analyzed by HPLC with electrochemical detection.

Results: Irrespective of the dose, almost none of the catechins originally contained in the green tea extract could be detected in plasma samples. Only catechin appeared at very small amounts in the blood samples. However, seven unidentified peaks (U1-U7) most likely presenting metabolites of the catechins applied were detected in plasma samples (fig. 1).

Fig. 1: Representative HPLC chromatograms of a plasma sample 2.5 h after i.r. application of 50 mg/kg KM Polyphenon 60(A), same sample spiked with standard (1 µmol of each individual catechin/l plasma, B), and the pure standard solution (C); U = unknown, C = catechin, EC = epicatechin, ECG = epicatechingallat, EGC = epigallocatechin, EGCG = epigallocatechingallat, GC = gallocatechin



Conclusions: Because individual catechins can be measured in plasma samples after intraduodenal application of Polyphenon 60 in dariy cows (results not schwon), the presented results clearly show that catechins are extensively metabolized by rumenal microorganisms. The unidentified peaks found in plasma samples most likely present substances resulting from bacterial break-down of the original catechins of the green tea extract.

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70. Effects of supplementing plant derived alkaloids on ruminal fermentation during normal and subacute ruminal acidosis conditions in RUSITEC

Effekte von Pflanzenalkaloiden auf die Pansenfermentation unter normalen und azidotischen Bedingungen im Rusitec-System

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Question: Subacute ruminal acidosis (SARA) is a fatal digestive disorder of high-producing cattle. Extensive research efforts have been made to develop feeding strategies that can help in minimizing the severity of SARA (1). In ruminant nutrition, the role of herbal components commonly known as phytobiotics has recently been recognized as rumen fermentation modulators (2). Thus, their effectiveness with respect to SARA seems plausible but has not been investigated so far. The objective of this study was to investigate the effect of supplementation of two different phytobiotics during normal and SARA conditions *in vitro*.

Methods: A RUSITEC-experiment was conducted in 3 identical runs, each using rumen innocula from 3 different cattle. The treatments were arranged as a 2 x 3 factorial design (2 incubation conditions x 3 diets), resulting in total of 6 treatment combinations (n=6). The incubation conditions were NORMAL with the target pH at ~6.7 and SARA with pH at ~5.5. SARA condition was accomplished by increasing dietary concentrate proportion (from 50 to 65% of DM) and modifying McDougall's artificial saliva. The diets were not supplemented (Control) or supplemented with two different plant derived alkaloids (PDA), containing quaternary benzophenanthridine and *protopine*_alkaloids as active substances, PDA1 at 0.08% of DM or PDA2 at 0.5% of DM. Each experiment lasted for 12 d with the last 5d serving as measurement period. Incubation fluid was collected for short chain fatty acid (SCFA) analysis in GC. Nutrient degradation was determined from the differences between the experimental feeds and the pooled feed residue samples after 48 h of incubation. Means were subjected to analysis of variance by MIXED procedure of SAS.

Results: Both PDA supplementations enhanced ($P < 0.05$) the concentration of SCFA compared to controls during NORMAL condition (Control = 90.8 mmol/L; PDA1 = 100.7 mmol/L; PDA2 = 103.6 mmol/L) but not during SARA conditions (Control = 73.0 mmol/L; PDA1= 79.3 mmol/L; PDA2 = 74.3 mmol/L; $P > 0.05$). Also, PDA supplementation enhanced ($P < 0.05$) propionate (Control = 18.4 mmol/L; PDA1 = 20.1 mmol/L; PDA2 = 20.5 mmol/L) and valerate concentration. In addition, PDA2 enhanced ($P < 0.05$) butyrate concentration but only during NORMAL conditions. Supplementation with PDA1 improved ($P < 0.05$) degradation of crude protein, in line with the increased concentration of iso-fatty acids, but only during NORMAL condition. However, during SARA conditions, PDA2 supplementation lowered ($P < 0.05$) degradation of non-fiber carbohydrates (NFC) by 13%. When calculated as a SCFA formation per each g of OM degraded, PDA supplementation improved fermentation efficiency (Control = 5.9 mmol SCFA/g OM degraded; PDA1 = 6.4 mmol SCFA/g OM degraded; PDA2 = 6.1 mmol SCFA/g OM degraded).

Conclusions: PDA supplementation modulated ruminal fermentation by increasing SCFA production and improving propionate formation and fermentation efficiency under normal ruminal condition. The decreased NFC degradation by PDA2 supplementation suggests a lowered degradation of starch during acidotic conditions, hence, potentially indicating beneficial effects in mitigating the severity of SARA.

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71. Effect of essential oil supplementation on rumen fermentation characteristics in rumen fistulated cows

Effekte einer Amylase und Proteasesupplementierung auf den ruminalen Trockensubstanzabbau bei trockenstehenden Holstein Kühen unter einer maisbasierten Fütterung

Deml M., Fahn C., *Windisch W. – Freising

Question: Increase in bypass starch and protein is important in highly producing dairy cows, as it supports milk production more efficiently. Therefore, it is important to modify rumen fermentation towards a better utilization of energy and protein. Essential oils have received much attention due to their potential to affect microorganisms [1]. In this context, the present study focused on effects of adding essential oils on rumen fermentation parameters and *in situ* dry matter degradability (DMD) of a total mixed ration (TMR) and its individual feedstuffs.

Methods: Six non-lactating rumen-fistulated cows were fed without or with dietary addition of essential oils (EO) (CRINA® Ruminants, DSM Nutritional Products Ltd., Basel, Switzerland; one g per head per day) in a Latin square design for two identical periods (2x3x2). A TMR consisting of 65% grass silage, 14% maize silage, 6% soybean meal, 6% rapeseed and 9% wheat was fed at amounts of 7.0 kg per head per day. Samples of TMR as well as of individual feedstuffs, were put into nylon bags (4 replicates) and were incubated for 0, 1, 2, 3, 4, 5, 6, 9, 12, 24, and 48 hours in order to determine rumen DMD according to the *in situ* method. In parallel, ruminal juice was collected up to 6 hours after feeding to determine rumen pH, volatile fatty acids (VFAs) and ammonia-N. Effective rumen dry matter degradability was calculated individually for each cow in both treatments, assuming a passage rate of 6% h^{-1} (EDMD6). Statistical analyses were carried out by 1-factorial ANOVA to determine significant differences between treatments (Control, EO addition).

Results: Rumen DMD (%) and EDMD6 (%) of the tested feedstuffs as well as rumen juice pH, VFAs (mg/ml) and NH_3-N (mg/L) at different incubation times (h) with or without essential oil (EO)

Feedstuff	EO addition	0-h	1-h	2-h	3-h	4-h	5-h	6-h	EDMD6
TMR	-	33.1	35.5	37.8	39.2	41.6	45.1	45.6	58.1
	+	33.3	36.4	37.4	39.9	41.2	43.4	46.3	57.1
Grass silage	-	30.6	29.7^{b}	31.0	31.6	34.5	38.1	39.1	52.7
	+	29.3	31.0^{a}	31.5	32.8	34.1	35.7	38.4	51.4
Maize silage	-	50.6	51.4	51.8	51.2	52.7	54.6	54.7	61.7
	+	51.7	52.7	52.5	52.0	52.0	53.1	55.7	61.9
Soybean meal	-	29.2	32.5^{b}	35.8	37.9^{b}	40.8	45.1	49.3	69.6
	+	31.1	33.8^{a}	36.6	39.8^{a}	42.3	45.9	50.8	69.4
Rapeseed meal	-	22.5	27.4	29.6^{b}	32.2^{b}	32.9^{b}	36.3	40.7	57.5
	+	24.8	29.6	31.7^{a}	34.8^{a}	37.0^{a}	38.8	43.5	58.0
Wheat	-	30.9	57.3^{b}	68.5	70.9	77.1	79.3	80.7	82.9
	+	37.5	63.7^{a}	70.5	76.1	76.6	80.1	84.0	84.2
Rumen pH	-	6.74	6.65	6.65	6.53	6.55	6.65	6.69	
	+	6.85	6.60	6.70	6.56	6.67	6.74	6.63	
Rumen VFAs	-	6.91	8.96	8.70	9.56	8.50	7.99	6.64	
	+	6.44	9.28	7.20	9.45	8.13	7.27	6.75	
Rumen NH_3-N	-	65.9	393	237	381	320	108	86.0	
	+	88.2	408	232	402	248	101	94.0	

Conclusion: Essential oil supplementation did neither affect rumen DMD of the tested feedstuffs nor the parameters of rumen physiology.

1) GREATHEAD, H.(2003). Proc. Nutr. Soc. 62: 279-290

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72. Effects of a natural plant antioxidant premixture on milk yield, antioxidative status and the expression of genes involved in endoplasmic stress response in dairy cows

Einfluss eines natürlichen pflanzlichen antioxidativen Präparats auf Milchleistung, antioxidativen Status und die Expression von Genen für die Antwort auf Stress des endoplasmatischen Retikulums in der Leber von Milchkühen

Maurer V., Amsler Z., Stamer A., *Leiber F. – Frick

During the first weeks of lactation, all cows develop a negative energy balance which leads to metabolic stress with undesirable effects on liver metabolism. Recently, it has been shown that stress of the endoplasmic reticulum (ER) which among others is triggered by an inflammatory process and reactive oxygen species might play a key role in liver dysfunction and the related symptoms such as fatty liver or ketosis which are commonly developing during this phase. In this study, we investigated the hypothesis that feeding a natural plant antioxidant premixture is able to suppress the development of ER stress in the liver and to improve liver function in dairy cows.

Methods: Twenty six primi- and multiparous dairy cows (German Holstein) were divided into a control group (n=14) and a group receiving a natural plant antioxidant premixture consisting of 95% of green tea extract and 5% curcuma extract (Loxidan®, Lohmann Animal Nutrition, Cuxhaven) at a daily dose of 3.5 g from 3 wk ante partum to 9 wk post partum (p.p.). The average number of lactation in these groups was 2.8 ± 1.6 and 3.1 ± 2.2, mean ± SD, respectively. All cows received a total mixed ration. Blood samples were taken in wks 1, 3 and 5 from *vena caudalis mediana* after the morning milking before feeding. Liver biopsies were taken at wks 1 and 3 p.p. mRNA concentrations of hepatic genes were determined by qPCR. Data were statistically evaluated by the Linear Mixed-Effects Model of R (version 3.1.1). The model included treatment, week, lactation number and the treatment x week interaction as fixed factors and cow as random factor.

Results: The dry matter intake of the cows did not differ between the two groups of cows. However, the group receiving the plant premixture had a better milk performance (Δ milk yield: + 3.2 kg/d; p=0.047; Δ energy corrected milk: + 4.2 kg, p=0.005) from wks 2 to 9 of lactation than the control group. Cows of the group receiving the plant premixture moreover had a lower mRNA concentration of fibroblast growth factor-21, a key marker of ER stress, in the liver (-74%; p=0.006). mRNA concentrations of various other genes involved in ER stress response such as activating transcription factor 4 (-40%), BCL2-associated X protein (-20%), ER degradation enhancer, mannosidase alpha-like 1 (-25%), immunoglobulin heavy-chain binding protein (-39%) and X-box binding protein 1 (-41%) in the liver were also reduced in the group receiving the plant premixture; however, these differences were not significant (p>0.05). In line with a suppression of ER stress, liver triglyceride content tended to be decreased in the group receiving the plant premixture (-34%, p=0.1385). The concentration of non-esterified fatty acids (NEFA) in plasma was reduced in cows receiving the plant premixture (-27%, p=0.003) while plasma concentration of ß-hydroxybutyrate was not different between both groups. The concentrations of α-tocopherol, ß-carotene, thiobarbituric acid reactive substances and the total antioxidant capacity in plasma did not differ between both groups. Plasma retinol concentration was increased in the cows receiving the plant premixture (P=0.0026).

Conclusion: This study shows that feeding a plant premixture consisting mainly of green tea extract is able to suppress ER stress in the liver of dairy cows and to increase milk yield. The findings of an increased concentration of retinol in plasma, a decreased concentration of NEFA in plasma and the tendency towards a reduced liver triglyceride concentration indicate that liver function was improved in the animals fed the plant premixture. As the antioxidant status was not improved by feeding the plant premixture, it is likely that beneficial effects of the plant premixture were rather induced by direct effects of polyphenols from green tea on gene expression than by exerting antioxidative effects.

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73. Effect of essential oil supplementation on rumen fermentation characteristics in rumen fistulated cows

Effekt einer Supplementierung ätherischer Öle auf die Pansenfermentation in pansenfistulierten Kühen

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Question: Increase in bypass starch and protein is important in highly producing dairy cows, as it supports milk production more efficiently. Therefore, it is important to modify rumen fermentation towards a better utilization of energy and protein. Essential oils have received much attention due to their potential to affect microorganisms [1]. In this context, the present study focused on effects of adding essential oils on rumen fermentation parameters and *in situ* dry matter degradability (DMD) of a total mixed ration (TMR) and its individual feedstuffs.

Methods: Six non-lactating rumen-fistulated cows were treated without or with dietary addition of essential oils (EO) (CRINA® Ruminants, DSM Nutritional Products Ltd., Basel, Switzerland; one g per head per day) in a Latin square design for two identical periods (2x3x2). A TMR consisting of 65% grass silage, 14% maize silage, 6% soybean meal, 6% rapeseed and 9% wheat was fed at amounts of 7.0 kg per head per day. Samples of TMR as well as of individual feedstuffs, were put into nylon bags (4 replicates) and were incubated for 0, 1, 2, 3, 4, 5, 6, 9, 12, 24, and 48 hours in order to determine rumen DMD according to the *in situ* method. In parallel, ruminal juice was collected up to 6 hours after feeding to determine rumen pH, volatile fatty acids (VFAs) and ammonia-N. Effective rumen dry matter degradability was calculated individually for each cow in both treatments according to, assuming a passage rate of 6% h^{-1} (EDMD6). Data for each feedstuff and rumen parameter were subject to two-factorial ANOVA (EO (-/+) and animal (1-6)).

Results: Rumen DMD (%) and EDMD6 (%) of the tested feedstuffs as well as rumen juice pH, VFAs (mg/ml) and NH_3-N (mg/L) at different incubation times (h) with or without essential oil (EO)

Feedstuff	EO addition	0-h	1-h	2-h	3-h	4-h	5-h	6-h	EDMD6
TMR	-	33.1	35.5	37.8	39.2	41.6	45.1	45.6	58.1
	+	33.3	36.4	37.4	39.9	41.2	43.4	46.3	57.1
Grass silage	-	30.6	29.7b	31.0	31.6	34.5	38.1	39.1	52.7
	+	29.3	31.0a	31.5	32.8	34.1	35.7	38.4	51.4
Maize silage	-	50.6	51.4	51.8	51.2	52.7	54.6	54.7	61.7
	+	51.7	52.7	52.5	52.0	52.0	53.1	55.7	61.9
Soybean meal	-	29.2	32.5b	35.8	37.9b	40.8	45.1	49.3	69.6
	+	31.1	33.8a	36.6	39.8a	42.3	45.9	50.8	69.4
Rapeseed meal	-	22.5	27.4	29.6b	32.2b	32.9b	36.3	40.7	57.5
	+	24.8	29.6	31.7a	34.8a	37.0a	38.8	43.5	58.0
Wheat	-	30.9	57.3b	68.5	70.9	77.1	79.3	80.7	82.9
	+	37.5	63.7a	70.5	76.1	76.6	80.1	84.0	84.2
Rumen pH	-	6.74	6.65	6.65	6.53	6.55	6.65	6.69	
	+	6.85	6.60	6.70	6.56	6.67	6.74	6.63	
Rumen VFAs	-	6.91	8.96	8.70	9.56	8.50	7.99	6.64	
	+	6.44	9.28	7.20	9.45	8.13	7.27	6.75	
Rumen NH3-N	-	65.9	393	237	381	320	108	86.0	
	+	88.2	408	232	402	248	101	94.0	

Conclusion: Essential oil supplementation did neither affect rumen DMD of the tested feedstuffs nor the parameters of rumen physiology.

1) GREATHEAD, H.(2003). Proc. Nutr. Soc. 62: 279-290

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74. Effect of individual medicinal plants and mixtures thereof on *in vitro* ruminal fermentation

Effekt von Heilpflanzen auf die Pansenfermentation in vitro

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According to the traditional knowledge of the Indian Ayurveda health care the plants *Andrographis paniculata* (Acanthaceae), *Helicteres isora* (Sterculiaceae), *Achyranthes aspera* (Amaranthaceae), *Tinospora cordifolia* (Menispermaceae), *Azadirachta indica* (Meliaceae) and *Piper longum* (Piperaceae) are supposed to support the mammalian liver metabolism, energy supply and digestion. These plants contain secondary compounds, which may be effective in ruminal fermentation, e.g. in terms of strategically reducing protein degradation to ammonia and methane formation. A two-stage approach was carried out, an in-depth *in vitro* program with first screening and then medium-term incubation of selected plants alone, in combination, and together with a basal diet.

Methods: The basis of the selection of the plants was given by a commercial mixture of these six plants designed for animal nutrition (Herb-All™ LIVER, Life Circle Nutrition, Wangen SZ, Switzerland). As a carrier, hickory nut fibre (*Carya illinoinensis*) is included in this product as well. With the Hohenheim gas test (HGT), different dosages of the single plants (2 and 200 mg dry matter) and of the plant mixture (1, 2, 5, 10, 20 and 200 mg) were incubated in duplicate either alone or as a supplement to a basal diet (200 mg; hay:concentrate, 80:20). With an eight-fermenter rumen simulation technique (Rusitec), three single plants (300 mg/d, *A. paniculata, T. cordifolia, P. longum*) and all binary combinations of these plants (150 mg/d each) as well as the plant mixture (300 mg) were tested as a supplement to the basal diet (12 g/d). Test feeds were incubated with ruminal fluid/buffer mixture for 24 h in HGT and for 10 d in Rusitec. In total six HGT runs and six Rusitec runs were performed yielding n=6 per dietary treatment each. The variables assessed included methane and carbon dioxide production, pH, bacteria and protozoa number. With Rusitec also ammonia was assessed; and the analysis of short chain fatty acids and digestibility is still in progress. Data analysis for HGT was done separately for single plants alone, as a supplement and the plant mixture as a supplement to include seven to nine treatments per analysis. The data were subjected to analysis of variance using the MIXED procedure of SAS 9.3 considering plant species as fixed effect and incubation run as random variable. Multiple comparisons among means were performed with Tukey's method. The significance level was set to α=0.05.

Results: All single plants alone (except *P. longum*) as well as the plant mixture alone (i.e. incubated without the basal diet) in HGT decreased ($p<0.05$) the absolute methane formation, but also the carbon dioxide formation compared to the basal diet control (on average 3 vs. 8 mL CH_4; 15 vs. 37 mL CO_2), thus only reflecting a lower supply with fermentable organic matter with the plants compared to the basal diet. When supplemented to the basal diets, both single plants and plant mixture had no effect ($p>0.1$) on methane and carbon dioxide formation, bacteria and protozoa number (10^8 and 10^5/mL, respectively) and ammonia concentration (7 mmol/L), either in HGT or in Rusitec. The pH slightly decreased by 0.1 units with *T. cordifolia* supplementation in HGT compared to basal diet control, but pH increased by 0.1 units with *T. cordifolia* in Rusitec compared to the binary combinations of *T. cordifolia* with either *A. paniculata* or *P. longum*.

Conclusion: According to the present *in vitro* results, the plant supplements did not improve ruminal fermentation in dosages fed to cattle or in even higher dosages. The mode of action of these plants in regard to a potential enhancement of animal performance may be through metabolic pathways (post-absorptive). As there was also not the least adverse effect on ruminal fermentation, the plants and their mixtures are not restricted in their application as feed supplements for other purposes.

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75. Relationships between milk odd and branched-chain fatty acids and urine-based microbial protein synthesis indicators in dairy cattle supplemented with quebracho tannin extract

Beziehungen zwischen ungeradzahligen und verzweigtkettigen Fettsäuren in der Milch und Indikatoren für die mikrobielle Proteinsynthese im Harn bei mit Quebrachotanninextrakt supplementierten Milchkühen

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Microbial protein synthesis (MPS) accounts for on average 59% of the protein available for duodenal absorption in ruminants (1). Therefore, the estimation of MPS under a given condition is essential to optimize ruminants' nutrition (2). There is an on-going interest to find reliable internal biomarkers in milk, because samples are easy to collect and are readily available to handle and store. Milk odd and branched-chain fatty acids (OBCFA) are constituents of the membrane of rumen microorganisms and, hence, are expected to reflect changes occurring on rumen metabolism (3). The objective of this study was therefore to explore the potential of OBCFA as biomarkers for MPS.

Methods. Data of a feeding trial with dairy cows was used for this study. Fifty lactating Holstein Friesian cows were divided into two groups housed in a free stall barn. They had free access to drinking water throughout the experiment that lasted for six consecutive periods à 21 d (13 d adaptation and 8 d of feed, milk, and urine sampling). The basal diet was a total mixed ration (TMR; 65:35 forage to concentrate ratio; Control). Quebracho tannin extract (QTE) was supplemented to the basal diet at levels of 15 g or 30 g/kg dry matter to create diets QTE15 and QTE30 respectively. Urine spot samples were collected between days 14 and 21 of each period and analysed for *total purine derivatives (PD), creatinine, and nitrogen concentrations*. The PD-creatinine index and the nitrogen-creatinine index were calculated as the molar concentration ratio of PD or nitrogen to creatinine times the metabolic body weight (kg) Simultaneously, milk samples were collected to determine proportions *(g/100 g fat)* and yields *(g/d) of OBCFA*. The effects of QTE dosage on OBCFA proportions *(g/100 g fat)* and yields *(g/d)* and on urine *indicators were tested using PROC MIXED of SAS (version 9.4). A multivariate regression of urine indicators regressed on individual OBCFA proportions and yields was done using the MIXED procedure of SAS (version 9.4).*

Results. The addition of QTE did not affect milk yield and fat content. Proportions and yields of OBCFA via milk decreased at QTE15 and QTE30 compared to the control, with the exception of *anteiso* C15:0, *anteiso* C17:0, and *cis*-9 C17:1, where no differences were observed between the three dosage levels. The ratios of PD to creatinine, the ratio of PD to nitrogen, the PD-creatinine index and the nitrogen-creatinine index in urine were lower at QTE15 and QTE30 compared to the control.

Multivariate models to predict urine indicators (i.e. allantoin, nitrogen, PD, ratio of PD to creatinine, ratio of PD to nitrogen, PDC index and NC index) from milk OBCFA proportions and yields explained between 15 and 57% of the variation in the dataset of these parameters. However, the explanation of that variance attributed to milk OBCFA proportions or yields was low and ranged between 2 and 10%. *Iso* C17:0, C17:0 and *anteiso* C17:0 were the OBCFA most commonly retained in the models. In general, models developed from milk OBCFA proportions explained more variance in the dataset than when models were developed from milk OBCFA yields.

Conclusions. Milk OBCFA are poor quantitative indicators for rumen MPS when dairy cows are supplemented with tannins. The variable transfer rate of microbial OBCFA to milk and the expected changes in the species composition of the microbial consortium caused by tannins might be responsible for the weak relationships between milk OBCFA proportions or yields and concentrations or ratios of established microbial indicators in urine.

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76. Effects of different milk diets and quercetin feeding on parameters concerning antioxidative and health status in neonatal calves

Effekte unterschiedlicher Milchdiäten und oraler Quercetin-Supplementierung auf antioxidativen und Gesundheitsstatus bei neugeborenen Kälbern

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Neonatal vitality often is impaired and diseases are catalyzed by oxidative stress, which arises when reactive oxygen species are not properly counter-regulated after birth (1). Moreover, calves are prone to infections as their immune system is immature at birth, and adequate colostrum supply is of major importance to acquire passive immunity (2). The flavonoid quercetin is known to exert anti-inflammatory and antioxidative effects (3) that could enhance neonatal health status. Therefore, we hypothesized that quercetin feeding during the first wk of life might improve inflammatory and antioxidative status in neonatal calves and that quercetin feeding might be able to compensate for an inadequate colostrum supply.

Methods: Newborn male Holstein calves were assigned to two feeding groups and fed same amounts of either pooled colostrum (**C**) or an isoenergetic milk-based formula (**F**) with much less bioactive factors than in C during the first two d of life and milk replacer from d 3 to d 8. On d 2, groups were subdivided into a control (**CQ-**; **FQ-**; n=7, respectively) and a treatment group (**CQ+**; **FQ+**; n=7, respectively), the latter receiving quercetin aglycone (25 mg/(kg BW × meal)). Before the morning meal on d 1, 2, 4 and 7, basal blood samples were taken from the jugular vein to measure plasma concentrations of quercetin by HPLC with fluorescence detection as well as total protein, albumin and urea photometrically using commercially available kits. For characterization of antioxidative status, trolox equivalent antioxidative capacity (**TEAC**), ferric reducing ability of plasma, thiobarbituric acid reactive substances (**TBARS**) and prostaglandin-like substances (**F2-isoprostanes**) were analyzed in plasma by spectrophotometer. Liver biopsy was taken 2 h after morning meal on d 8 to determine mRNA abundances of markers for antioxidative status (catalase; glutathione peroxidase, superoxide dismutase) and inflammation (tumor necrosis factor, **TNFα**; serum amyloid A2, **SAA2**; C-reactive protein, **CRP**) using qRT-PCR. Plasma data were evaluated by Mixed Model Procedure and PCR date by General Linear Model of SAS with feeding, quercetin, day and their interactions as fixed effects.

Results: Rectal temperature and incidence of diarrhea were greater in F- than in C-fed calves ($P < 0.01$). In treatment groups, concentrations of quercetin increased in plasma from d 2 to d 3 and decreased afterwards ($P < 0.001$). Total protein was greater ($P < 0.001$) in C- than in F-fed calves due to immunoglobulin (Ig) intake. TEAC and TBARS were greater in C- than in F-fed calves ($P < 0.05$). Relative mRNA abundance of TNFα was greater in quercetin-fed groups ($P < 0.05$); CRP was greater ($P < 0.05$) and SAA2 tended to be greater ($P < 0.1$) in F- than in C-fed calves.

Conclusions: Absence of Ig in F groups was associated with higher body temperatures, greater incidence of diarrhea and increased hepatic mRNA abundances of acute phase proteins, which underlines the importance of colostrum feeding during the first days of life to support neonatal health. Results further indicate that during 1st wk of life quercetin supplementation of 50mg/(kg BW × d) barely affects the anti-inflammatory and anti-oxidative status in neonatal calves, although quercetin was absorbed in significant amounts in calves.

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77. Daily quercetin supplementation over a period of two weeks results in a moderate accumulation of total plasma flavonols in horse

Eine zweiwöchige Quercetinsupplementierung führt zu einer moderaten Akkumulation der Gesamtflavonole im Blutplasma von Pferden

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The flavonol quercetin has anti-inflammatory and other health-promoting properties. We have previously documented bioavailability of quercetin in horses after a single oral dose (1). In the present study we investigated effects of daily quercetin supplementation over a period of two weeks on plasma concentrations of quercetin and its metabolites.

Methods: Four adult healthy Warmblood horses with a mean body weight (BW) of 551 kg were used. Horses were offered hay *ad libitum*, concentrated feed individually (7 AM/PM), and eight hours access to pasture. During the pre-experimental phase (14 days) and also during the experiment the use of dietary supplements, apples or any form of medication was excluded. During the experiment, quercetin (10 mg/kg BW) was supplemented daily within the evening meals. Blood samples were collected from the jugular vein three times during the pre-experimental phase and five times (day 4, 8, 11, 14) after the start of the feeding experiment. Flavonols in plasma (quercetin, kaempferol, isorhamnetin) and feed were analyzed by HPLC with fluorescence detection. The time course of plasma flavonol concentrations was statistically evaluated by a 2-way ANOVA followed by Bonferroni post-hoc tests.

Results: The plasma concentration of total flavonols increased over the feeding period, resulting in significant higher values after 11 and 14 days compared to the initial value. The increase was mainly due to an increase of kaempferol as indicated by a significant higher value after 14 days of quercetin feeding (Fig. 1).

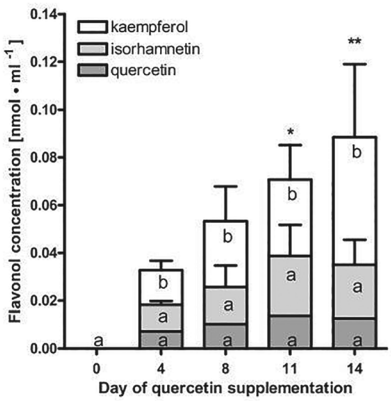

Figure 1: Plasma concentrations of total flavonols during 2 weeks of oral quercetin supplementation (10 mg/kg BW); data are means ± SEM (n = 4); *, ** total flavonol concentration differs significantly from day 0, $P < 0.05$ or 0.001, respectively; concentrations of individual flavonols bearing no common letter differ significantly

Conclusions: From the present study we can conclude, that daily quercetin supplementation for 14 days led to a very moderate accumulation of total flavonols in plasma mainly due to an increase of the concentration of the quercetin metabolite kaempferol. Because kaempferol was not detectable in plasma samples at the start of quercetin supplementation period (day 0) despite the same basic feeding regime, kaempferol in plasma most likely originates from unabsorbed quercetin after microbial dehydroxylation in the large intestine. (1) WEIN S, WOLFFRAM S (2013) Oral Bioavailability of Quercetin in Horses. J. Equine Vet. Sci. 33, 441-445. This study was supported by the Wilhelm Schaumann Stiftung, Germany.

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78. Effects of an *in vitro* challenge with enterotoxigenic *Escherichia coli* on jejunal epithelia from piglets of a feeding trial with probiotic supplementation

Effekte einer in vitro Challenge mit einem enterotoxischen Escherichia coli-Stamm auf Jejunumepithelien von Ferkeln aus einem Fütterungsversuch mit Supplementierung von Probiotika

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Probiotic supplementation with *Enterococcus faecium* NCIMB 10415 has been demonstrated to reduce diarrhea incidence in piglets (1, 2). Antisecretory effects in the intestine have also been shown with the probiotic *E. coli* Nissle 1917 under challenge conditions with pathogenic *Escherichia coli* strains *in vivo* and *in vitro* (3). To further analyze these effects, we used an *in vitro* infection model and examined effects of an enterotoxigenic *E. coli* strain on jejunal epithelial barrier and transport functions of probiotic-treated and control piglets.

Methods:

Sixteen 29-d-old unweaned piglets from sows fed with an *E. faecium*-supplemented or control diet were killed. The mid-jejunum was stripped off its muscle layers and mounted into conventional Ussing chambers. Part of the epithelia was challenged on the mucosal side with an enterotoxigenic *E. coli* O149:K91:K88 strain (ETEC, 10^8 CFU/ml). Barrier and transport properties were assessed by electrophysiological experiments. The barrier function was further characterized by molecular techniques and by fluxes of fluorescein as a marker of paracellular permeability. Variance analyses with post-hoc Scheffe tests were performed for statistical evaluation of the data.

Results:

The incubation with ETEC *in vitro* induced an increase in transepithelial resistance (R_t) within the first two hours after addition compared to control epithelia (60 min: 45%, 120 min: 81% increase). Concomitantly, the fluorescein fluxes were reduced in the ETEC-treated epithelia indicating effects on barrier function. An increase in short-circuit current (I_{sc}) indicated changes in ion transport in the presence of ETEC in part of chambers; however, there was no overall significant effect on I_{sc} (60 min: 12%, 120 min: 9% increase). Nonetheless, the response of I_{sc} after stimulation with PGE_2- or glucose was generally reduced in epithelia treated with ETEC. The latter seemed to be less pronounced in probiotic-treated animals. In ETEC-treated epithelia the response to PGE_2 was reduced by 53% and 34% in control resp. probiotic-fed animals.

Conclusions:

The addition of ETEC *in vitro* induced a barrier-enhancing effect within the first two hours after addition and reduced both the electrogenic glucose absorption and the PGE_2-induced secretion across porcine intestinal epithelia. The pre-feeding of mother sows with *Enterococcus faecium* did not significantly alter the response of piglet jejunum to ETEC in the present model.

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79. Expression of the natural killer cell receptor NKG2D on jejunal epithelium and the impact of *Bacillus cereus* var. Toyoi on the absolute cell count of intraepithelial immune cell populations in piglets

Expression des Natürliche Killerzellen Rezeptors NKG2D auf jejunalem Epithel und der Einfluss von Bacillus cereus var. Toyoi auf die absolute Zellzahl der intraepithelialen Immunzellpopulationen bei Ferkeln

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Intraepithelial lymphocytes (IEL) form a first line of defence of the immune system. They are suited to communicate with the adjacent enterocytes (1). It can be speculated that probiotics like *B. cereus* var. Toyoi have an impact on IEL activation. This study was conducted to determine the effect of *B. cereus* var. Toyoi on the absolute cell count of IELs and to find a correlation between IEL frequency and the expression of the natural killer receptor NKG2D in jejunal epithelium of piglets.

Methods: A total of 40 piglets from 8 landrace sows were used. Sows in the *Bacillus* group (n=4) received 3.5 x 10^8 CFU/g *B. cereus* var. Toyoi from 90 days a.p. until weaning of piglets. Piglets in the probiotic group received creep feed from day 14 with 1.5 x 10^9 CFU/g. Five piglets per group were euthanized and intestinal tissue samples were taken before and after weaning. Via flow cytometry the frequencies of jejunal IEL-populations were measured via FACSCalibur (Becton Dickinson, Heidelberg). Absolute numbers of T cells (CD3) were counted on paraffin tissue sections in parallel. These cell numbers were used to extrapolate the flow cytometry data of the diverse IEL-populations. The mRNA expression of NKG2D was determined by qRT-PCR in jejunal epithelial cells as well as in IEL populations isolated via magnetic cell sorting. Means were compared by one-factorial analysis of variance using SPSS (version 21.0, Chicago, USA).

Results: Piglets fed with *B. cereus* var. Toyoi showed a lower count of CD3+ cells ($p = 0.01$). The calculated cell counts of other IEL populations revealed a lower count in the *Bacillus* group for leukocytes shown by the quantity of CD45+ cells ($p = 0.01$) and CD8aß T cells ($p < 0.01$), which are reported to be cytotoxic T cells (2) (Table 1). Gene expression analysis showed NKG2D expression on gd T cells (21.52 %) isolated using an antibody against TcR1. Isolation of T cells with two different T-cell markers revealed different results for the NKG2D expression. The cells sorted with CD3 reached 29.84 % in comparison to 2.54 % in those sorted with CD5. A positive correlation was detected between NKG2D expression in jejunal epithelium and the lymphocyte (CD45, $p = 0.011$) and CD8α/γδ- cell count ($p = 0.033$). However, there was no correlation observed with CD8/γδ cells.

Table 1: Calculated absolute cell counts [cells / 100 Enterocytes] of various IEL populations in jejunal epithelium (n=5)

	Control				***B. cereus*** var. Toyoi				SEM	p value	
Age [days]	17	32	34	39	17	32	34	39		Group	Age
CD45+[1]	17.2	6.57	2.67	8.59	1.39	3.35	7.86	4.92	1.339	0.010	0.090
CD3+	24.1	16.9	17.8	19.0	9.45	12.2	12.3	11.3	1.426	0.003	0.754
CD5+/γδ-	15.8	8.36	8.09	10.3	4.50	5.69	6.51	5.73	0.919	0.001	0.170
CD8ß+/CD16-	11.7	6.36	7.18	7.97	1.94	3.17	4.27	3.32	0.734	0.000	0.275
CD8α+/γδ-	31.9	14.0	7.09	16.1	17.9	6.54	10.6	11.1	2.125	0.075	0.002
CD2+/CD5-	24.8	12.8	7.12	13.9	26.8	15.8	13.4	17.6	2.599	0.474	0.079
CD16+/CD8ß-	19.4	9.49	4.63	10.4	19.7	12.4	11.1	13.7	1.813	0.360	0.044
CD11R1+	4.15	0.86	1.68	2.06	3.92	1.47	0.70	1.76	0.407	0.771	0.006
CD8+/γδ+	6.63	5.15	6.38	5.93	4.93	4.09	3.97	4.25	0.592	0.195	0.775
CD5-/γδ+	1.86	1.67	2.22	1.89	3.02	2.06	0.86	1.80	0.391	0.940	0.704

[1] CD45+ describes the frequency of leucocytes in all cells isolated from the epithelium. Within the selected leucocyte population, the distribution and relative frequency of the remaining IELs was determined. CD45, leucocytes; CD3, CD5, γδ, T lymphocytes; CD2, T lymphocytes and NK cells (CD2+ CD5-); CD8, cytotoxic T lymphocytes, CD16, CD11R1, NK cells.

Conclusion: The lower frequency of lymphocytes and esp. of T cells in the *Bacillus* group suggests a delaying effect of the probiotic on adaptive immune system development. QRT-PCRs have shown NKG2D expression on γδ T cells for the first time in pigs, revealing their potential to communicate with stressed enterocytes and to initiate an intestinal immune response. The expression of NKG2D could not be ascribed to one distinct IEL population in vivo. It appears that additionally to the expression on gd T cells, NKG2D can be expressed on CD8ab T cells after activation (engagement of the CD3 complex).

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80. **Effect of supplementation of two different ß-mannanase sources on broiler performance**

Effekte der Zulage zwei verschiedener ß-Mannanasen auf die Leistung von Masthähnchen

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Mannan polysaccharide is a highly anti-nutritional factor in poultry nutrition. The negative effects of ß-mannan can be, however, overcome by the addition of ß-mannanases to the feed of monogastrics. To the best of our knowledge, the efficacy of different ß-mannanase sources in poultry has not been compared yet. Thus, the objective was to compare the effect of supplementation of two different ß-mannanase sources on broiler performance in an established challenge model (1).

Methods: For the negative control (NC), a maize-soybean meal-based diet with 1.5% guar gum was formulated to exceed dietary recommendations (CVB**, 2008) by about 5% (22% CP; 13.1 MJ ME/kg). This NC diet was fed as is or supplemented with four levels of a *Trichoderma*-ß-mannanase (TMan) (analysed at 3700, 10800, 27600 or 50400 MNU***/kg feed, respectively) or a commercially available *Bacillus*-ß-mannanase product (BMan) at the highest recommended dosage (analysed at 3900 MNU/kg feed). A positive control diet (PC) was formulated by using maize starch instead of guar gum in the NC. After a 7 days conventional rearing period, broilers (male Cobb 500) were switched to pelleted experimental diets for the following 14 days. Each of the seven diets was fed to eight replicate pens of 12 birds. Birds had continuous access to feed and water. Body weight for each pen was recorded on days 7 and 21 post hatch. Feed intake was measured on a pen basis. Total BW gain and mortality corrected feed conversion ratio were calculated. Data were analysed by one-factorial ANOVA, treatment differences were assessed by Tukey test.

Results: ß-Mannans from guar gum in the NC diet had a pronounced negative effect on broiler performance (Table). The supplementation of BMan to the NC had a positive effect on broiler performance ($P < 0.05$), but birds were not able to achieve the level of performance of the PC and NC+TMan treatments ($P < 0.05$). All four NC+TMan treatments achieved performance similar to the PC without a dose-response relationship. Performance for all NC+TMan treatments was significantly increased as compared to the NC and NC+BMan treatments (Table).

Table: BW gain (BWG), feed intake (FI) and mortality corrected feed conversion ratio (FCR).

Diet	BWG	FI	FCR
	g/d	g/d	feed/gain
Positiv control diet	57.7^{c}	79.7^{b}	1.38^{a}
Negative control diet (NC)	36.8^{a}	70.3^{a}	1.91^{c}
NC+3900 MNU BMan	51.5^{b}	78.2^{b}	1.52^{b}
NC+3700 MNU TMan	57.6^{c}	81.5^{b}	1.41^{a}
NC+10800 MNU TMan	57.5^{c}	80.4^{b}	1.40^{a}
NC+27600 MNU TMan	57.7^{c}	80.6^{b}	1.39^{a}
NC+50400 MNU TMan	57.5^{c}	79.2^{b}	1.38^{a}
*$^{a-c}$Means within columns without common superscript differ significantly ($P < 0.05$)			

Conclusions: At the same level of supplementation (~3800 MNU/kg), TMan was superior to BMan. The reason for this might have been differences in resistance against proteolytic enzyme activity, pH optimum of enzyme, substrate specificity etc. The lowest supplementation level of TMan (3700 MNU/kg) fully restored broiler performance to the level of birds fed PC diet. Further studies are required at lower supplementation levels of TMan.

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*BASF SE, E-ENE, 68623 Lampertheim, Germany; **CVB = Centraal Veevoeder Bureau, The Netherlands; ***Mannanase unit (MNU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one nmol mannose from mannan in one second under the assay conditions.

81. Efficacy of phytase supplementation in uncoated and coated form in male broiler chickens

Effektivität einer Phytaseergänzung in ungecoateter und gecoateter Form bei männlichen Broilern

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Introduction: Enzymes like all proteins are heat sensitive. However, as the biggest proportion of feed is pelleted, heat stability of enzymes is very important. Coating is one option to increase survival rate during the pelleting process. Nevertheless, coating might affect the release of the enzyme in the intestine and therefore its efficacy. The aim of this study was to evaluate the effect of an uncoated and coated phytase on performance and bone mineralization in broiler chickens. **Material and methods:** 100 one-day-old male broiler chickens were housed in balance cages with 2 birds per cage and allotted to 5 treatments. The feeding period included starter (1 to 14 d of age) and grower (15 to 35 d of age) diets as mash feed according to the recommendations, except that phosphorus was reduced in order to allow for a response to phytase addition as uncoated or coated product at dose levels of 250 or 500 FTU/ kg feed. Treatments were: control (A),250 FTU/kg uncoated (B), 500 FTU/kg uncoated (C), 250 FTU/kg coated (D) and 500 FTU/kg coated (E) of an *E.coli* derived 6-phytase (Enzy Phostar, Lohmann Animal Nutrition, Cuxhaven). The digestible phosphorus content of the diets was calculated to be 2.5 g kg^{-1} of starter and 2.2 g kg^{-1} of grower diet. Investigated parameters were body weight, body weight gain, feed intake, FCR as well as ileal digestibility (crude ash, phosphorus, calcium), and left tibia bone mineralization at the end of the trial period. **Results and discussion:** Due to the inadequate phosphorus supply birds fed diets without phytase addition reached approximately 80% of the intended body weight gain given by the breeding company. Feeding diets with phytase addition led to significantly increased body weight gain (+14.1 %) and significantly improved (10.8%) FCR compared to birds fed without phytase. Based on apparent ileal P-digestibility it could be shown that the coated phytase was numerically (250 FTU kg^{-1} of diet) or significantly (500 FTU kg^{-1} of diet) better in comparison to the uncoated preparation. Moreover, animal performance correlated significantly with the dose level irrespective of the form. Ileal digestibility and tibia bone deposition of phosphorus, crude ash and calcium also showed a dose depending effect. **Conclusion:** Based on the apparent ileal digestibility and the tibia mineralization the coated phytase at the dose levels of 250 and 500 FTU $kg^{-1}$of diet was similar or superior to the uncoated form. Moreover, the benefits of uncoated or coated phytase increased with increasing dose level.

Table: Influence of treatment on ileal digestibility and tibia mineralization. [a,b] Values in the same row with no common superscript are significantly different (P£0.05).

	Treatment					P value
	A	B	C	D	E	
ID Crude ash	46.89 [a]	50.80 [ab]	54.07 [b]	50.10 [ab]	53.64 [b]	<0.006
ID Phosphorus	42.95 [a]	52.41 [b]	60.29 [c]	55.65 [bc]	65.93 [d]	<0.001
ID Calcium	52.75 [a]	58.38 [abc]	62.02 [bc]	57.04 [ab]	63.84 [c]	<0.001
Tibia bone weight (g)	10.1	10.9	11.6	11.1	10.7	0.211
Tibia fat free dry matter (%)	36.88	37.98	37.10	37.45	37.07	0.937
Crude ash (g/kg of tibia DM)	262.0[a]	308.2 [b]	313.1 [b]	313.1 [b]	311.9 [b]	<0.001
Phosphorus (g/kg of tibia DM)	34.6 [a]	40.5 [b]	43.3 [b]	42.4 [b]	49.6 [c]	<0.001
Calcium (g/kg of tibia DM)	92.8 [a]	106.4 [b]	110.5 [bc]	108.7 [bc]	113.1 [c]	<0.001

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82. Effect of a mono-component protease on ileal digestibility and protein utilization of a grower diet in meat type chicken

Wirkung einer Mono-Komponenten-Protease auf ileale Verdaulichkeit und Proteinverwertung einer Growermischung bei Masthähnchen

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A certain amount of protein passes through the GI tract without being completely digested (1), which presents an opportunity for the application of exogenous protease. Mostly, in earlier studies complexes of enzymes were being investigated. Therefore, the monitored effect cannot be attributed to a single enzyme. A commercially available exogenous mono-component serine protease (Ronozyme® ProAct) revealed inconsistent impacts on protein and amino acid (AA) digestibility. The objective of the current study was, to evaluate this enzyme in a grower diet by assessing both parameters of ileal digestibility and protein utilization in growing meat type chicken.

Methods: Day-old male boiler chicks (ROSS 308) were raised in floor pens for 30 days under standardized management conditions. On day 31, a total of 36 average weighed birds were selected for the N-balance study with 12 birds per treatment which were randomly allotted and housed individually in metabolic cages. Following 5d adaptation, excreta were collected for 5d. Isonitrogenous diets based on corn, wheat, SPC, potato protein, fish meal) with uniform AA ratios were: Control diet (CD) and two diets with graded enzyme level (I:15,000 PROT units/kg; II:30,000 PROT units/kg). Titanium dioxide (0.3%) was used as an indigestible marker for measures of apparent ileal digestibility (AID). One day after finishing the N balance study and *ad libitum* supply of the diets, all birds were euthanized by CO_2 inhalation. Ileal contents were collected 2h after morning feeding and frozen for further analyses by ion-exchange chromatography. For protein quality evaluation, the model parameters were adapted from (2) to assess protein quality parameter (*b*) according to (3). One-way ANOVA (SPSS software package) and Tukey test were applied to identify statistical significance of differences ($p<0.05$).

Results: AID of crude protein (CP) was significantly improved by both of the graded protease levels compared to CD. AID of individual AA also responded gradually according to the protease level. This enhancement of AID was found for each of the amino acids under study, but statistically insignificant. Otherwise, the model parameter (*b*) as a complex measure of dietary protein utilization (3) including AID was not improved by protease supplementation ($p>0.05$).

Nutrient	Control	Enzyme Level-I	Enzyme Level-II	P
Enzyme added (PROT units/kg)	0	15,000	30,000	
Enzyme activity (PROT units/kg)*	0	15,870	33,070	-
AID (%) CP	78.64[a]	82.08[b]	83.37[b]	<0.001
Lys	83.70[a]	86.42[a]	87.95[a]	0.162
Met	83.30[a]	86.47[a]	88.47[a]	0.143
Cys	64.04[a]	69.56[a]	73.62[a]	0.083
Thr	71.38[a]	75.97[a]	78.98[a]	0.110
Val	78.85[a]	81.60[a]	84.74[a]	0.095
Ileu	78.54[a]	81.57[a]	84.33[a]	0.108
Leu	81.75[a]	84.84[a]	86.63[a]	0.134
b-value	366[a]	368[a]	356[a]	0.289

*analyzed; [a,b] different superscript letters indicate significant differences ($p<0.05$).

Conclusions: The added protease improved AID of CP significantly, but only numerical for individual AAs. However, the observed dietary protein quality was unchanged. In consequence, this contrast scrutinizes the nutritional significance of the observed AID responses because the AID of Met as limiting dietary AA also tended to be increased, but response on protein quality was missed.

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83. Influence of a yeast cell wall product on the performance parameters and the composition and activity of the intestinal microbiota of broiler chickens

Einfluss eines Hefezellwandproduktes auf die zootechnische Leistung sowie die Zusammensetzung und Aktivität der intestinalen Mikrobiota bei Masthühnern

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Mannan oligosaccharides (MOS) and (1→3), (1→6)-β-D-glucans are main components of the yeast cell wall and can positively influence the performance parameters and the intestinal microbiota of broiler chickens (1, 2). However, the results of the different studies are inconsistent. Differences seem to be dependent on the origin of the yeast cell wall sources, production processes and amounts of β-glucans and MOS in the different products (3). Moreover, studies regarding the effect of yeast cell wall components on the microbiota in the crop are rare. For that purpose a study was conducted to determine whether a yeast cell wall product can exert a dose-dependent impact on performance parameters and the composition and activity of the microbiota in the crop, ileum and caeca of broiler chickens.

Methods: A total of 669 male broiler chickens (Cobb Germany Avimex GmbH) were used in two consecutive trial runs. Four experimental groups were fed the yeast cell wall product ImmunoWall® (MIAVIT GmbH, Essen, Germany) in rising concentrations (0.05 %, 0.10 %, 0.20 % and 0.30 %) over the whole fattening period. The fifth experimental group served as control. All animals received a starter (d 1-14) and grower mixture (d 15-35) based on soybean meal, maize and wheat. The performance parameters were recorded weekly. On day 35, one broiler chicken from each experimental unit was slaughtered and digesta samples from the crop, ileum and caeca were taken. Via qPCR (Stratagene Mx3000P QPCR System, Agilent Technologies, USA) the lactobacilli, bifidobacteria, enterobacteria and *Escherichia/Hafnia/Shigella* spp. were quantificated as well as the bacterial metabolites. The collected data were analysed by using a one-factorial analysis of variance or a Kruskal-wallis-test as well as a polynomial contrast analysis (SPSS Statistics 21, Chicago, USA).

Results: A positive dose-dependent effect was observed for the lactobacilli in the digesta of the crop and ileum ($p = 0.010$ and $p = 0.002$). Similar trends were found regarding the lactate and acetic acid concentration in the crop and ileum, while the yeast cell wall product had no impact on the composition of the microbiota in the caeca.

Table: Performance parameters [g] of the five experimental groups (mean ± SD); n = 8

	Yeast cell wall product					p-value		
	0 %	0.05 %	0.10 %	0.20 %	0.30 %	ANOVA	linear	quadratic
BW d 7	159 ± 5.57	161 ± 4.91	158 ± 8.29	159 ± 4.93	162 ± 5.65	0.751	0.551	0.382
FI d 1-7	159 ± 11.8^{ab}	150 ± 6.02^{b}	147 ± 7.60^{b}	157 ± 6.01^{ab}	165 ± 11.1^{a}	0.002	0.013	0.002
FCR d 1-7	1.35 ± 0.11	1.26 ± 0.06	1.27 ± 0.07	1.35 ± 0.07	1.38 ± 0.13	0.081	0.121	0.093
BW 14	443 ± 20.1	447 ± 20.5	444 ± 20.1	451 ± 22.6	464 ± 16.5	0.220	0.029	0.434
FI d 8-14	345 ± 25.7	347 ± 16.1	351 ± 18.0	352 ± 19.5	352 ± 16.0	0.914	0.415	0.647
FCR d 8-14	1.22 ± 0.04	1.22 ± 0.03	1.23 ± 0.03	1.21 ± 0.07	1.17 ± 0.07	0.286	0.180	0.220
BW d 21	946 ± 37.0	952 ± 27.6	944 ± 39.0	958 ± 47.5	979 ± 45.7	0.430	0.089	0.431
FI d 15-21	678 ± 34.3	682 ± 23.8	706 ± 62.5	689 ± 48.1	690 ± 38.2	0.754	0.639	0.419
FCR d 15-21	1.35 ± 0.05	1.35 ± 0.04	1.41 ± 0.08	1.36 ± 0.07	1.34 ± 0.05	0.211	0.728	0.083

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

Conclusion: The feed additive showed dose-dependent effects on the performance parameters and the intestinal lactobacilli of broiler chickens. The Additive could be used to have a positive impact on the composition and activity of the intestinal microbiota as well as the performance parameters in the first three weeks of age. It is possible that the carbohydrates of the yeast cell wall were fermented by the lactobacilli in the upper intestinal tract and thus could indicate a better intestinal health.

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84. Effect of a combination of Benzophenanthridine and Protopine alkaloids on nitrite production of lipopolysaccharide challenged macrophages and on intestinal lesions of broilers challenged with *Clostridium perfringens*

Effekt einer Kombination aus Benzophenanthridin- und Protopinalkaloiden auf die Nitritproduktion von mit Lipopolysacchariden infizierten Makrophagen und auf intestinale Läsionen von mit Clostridium perfringens infizierten Broilern

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Clostridium perfringens (*C.p.*) is known to be a contributing factor in developing necrotic and bacterial enteritis in broilers, causing severe economic losses. Symptoms in this matter occur in loss of performance, increased feed conversion ratios and mortality. Furthermore, increased lesion scores in the intestinal tract indicate a clear sign of inflammatory processes. The aim of the study was to determine the effect of standardized blends of plant alkaloids with active ingredients from the groups of quaternary Benzophenanthridine and Protopine alkaloids (QBA+PA, Sangrovit® (S®)) supplemented in feed on (T1) nitrite production of lipopolysaccharide (LPS)-challenged macrophages as a general indicator for inflammatory processes and (T2) on lesion scores of broilers challenged with *C.p.*

Methods: The two trials (T1 and T2) were conducted with broiler chicks (mixed sex) in Petersime batteries. The corn-soy based diets met nutritional requirements according to (1). In T1, following treatments with a total of 40 broiler chicks (n=10) were established: 1.1) Control (no supplementation), 1.2) QBA+PA 5 (S® 5 g/t feed), 1.3) QBA+PA 10 (S® 10 g/t feed), 1.4) QBA+PA 25 (S® 25 g/t feed). Feed and water were provided *ad libitum* and QBA+PA feeding started at day of hatch. At 3 weeks of age, broilers were euthanized and Sephadex-elicited macrophages were harvested from exudate of the abdominal cavity of broilers and cultured as monolayers in 24-well plates. Cultures (n=5) were exposed to 1µg/ml of *E. coli (*strain 055:B5, Sigma Chemical Co.) LPS (+LPS) for 24 hours. The 5 remaining cultures per group were unchallenged (-LPS). The supernatants were then collected and tested for nitrite activity using Griess reagent. For T2, 120 broiler chicks were fed 3 different diets from day of hatch (n=40): 2.1) Control (no supplementation), 2.2) QBA+PA 10 (S® 10 g/t feed), 2.3) QBA+PA 25 (S® 25 g/t feed). At 7 days of age, 20 chicks per treatment were orally challenged with *C.p.* (1 x 10^8 CFU/ml, suspended with sterile phosphate buffered saline (PBS)), whereas 20 chicks received an oral gavage of pure PBS (non-challenge). Intestinal lesion scoring was realized at 7 and 14 days post-infection (dpi) on 10 broilers per treatment, respectively. Intestinal necropsy for lesion scores was performed by two independent researchers using the following scoring criteria: 0 = no gross lesions; 1 = thin walled or friable intestine; 2 = focal necrosis or ulceration; 3 = large patches of necrosis; 4 = severe extensive necrosis. Statistical analyses were realized by SAS. For nitrite production data were evaluated by ANOVA using the GLM procedures. For comparison of individual effects of LPS challenge, least square means were chosen. Significance was established at P≤0.05.

Results: In T1, -LPS procedure showed lower constitutive nitrite levels in the culture supernatants for all dietary QBA+PA treatments. For T2, lesion scores of birds in both of QBA+PA treatments were decreased in a dose-dependent way compared to control. By 14 dpi, birds showed signs of recovery, since the lesion scores in all treatment groups were lower than at 7 dpi.

Treatment	T1 - Nitrite production (µM)		T2 - Lesion Score			
	+LPS	-LPS	Unchallenged (7dpi)	Challenged (7dpi)	Unchallenged (14dpi)	Challenged (14dpi)
Control	8.55 (±1.77)	2.60^{a} (±0.38)	0.00^{c} (±0.16)	2.10^{a} (±0.16)	0.00^{c} (±0.13)	1.83^{a} (±0.14)
QBA+PA 5	8.85 (±1.51)	1.75^{ab} (±0.34)				
QBA+PA 10	9.30 (±1.69)	1.93^{a} (±0.38)	0.00^{c} (±0.16)	1.20^{b} (±0.16)	0.00^{c} (±0.13)	1.05^{b} (±0.14)
QBA+PA 25	5.30 (±1.63)	0.82^{bc} (±0.34)	0.00^{c} (±0.16)	0.45^{c} (±0.16)	0.00^{c} (±0.13)	0.35^{c} (±0.14)

^{abc} indicate significant difference (≤0.05)

Conclusion: The findings of T1 confirm that QBA+PA downregulated macrophage activity, as described by Niewold (2). The reduction of intestinal lesions in *C.p.* challenged birds (T2) supplies additional insights into mechanisms altered by QBA+PA, making those substances appropriate candidates contributing to the prevention of *C.p.* infection in broilers.

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85. Nutritional modulation of insulin and incretin homeostasis by butyrate in broiler chickens

Nutritive Beeinflussung der Insulin- und Inkretin-Homeostase bei Broilern

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Since broiler chickens have high growth and muscle protein synthesis rates, regulated significantly by insulin, investigating their insulin homeostasis and exploring possible ways in nutrition for affecting its regulation can be of special importance. It was recently found that orally applied butyrate, which is widely used as a feed additive in poultry nutrition, could influence insulin signaling of chickens on a tissue-dependent manner, selectively up-regulating insulin receptor β subunit in skeletal muscle (1). Insulin release is primarily regulated by incretin hormones, e.g. by GIP (Glucose-dependent Insulinotropic Peptide) and GLP-1 (Glucagon-like Peptide 1) in mammals (2), however, their regulatory role is not fully elucidated in chicken. In the present study, it was aimed to investigate the function of incretins in the regulation of insulin homeostasis in chicken, as well as to focus on its modulation by nutritional factors, such as butyrate.

Methods: Newly hatched male Ross 308 type broiler chickens (n=7/group) were fed with conventional starter diet according to the Ross standard. Growth parameters completely matched the requirements of the Ross 308 technology over the entire trial. On day 24, following an overnight feed deprivation, animals were acutely challenged with a single intra-ingluvial sodium butyrate bolus (0,25; 1,25 g/kg BW) or with physiological saline solution to serve as control. This way of butyrate application provides an already approved *in vivo* model to study the acute biochemical effects of orally administered butyrate (1). Blood samples were drawn from the brachial vein prior to the treatment (0. min) and sampling was repeated three times (10., 30., 60. min) after the butyrate administration. Plasma concentrations of insulin, GIP and GLP-1 were determined by ELISA and blood glucose concentrations were assessed by a spectrophotometric assay. Data were compared by one-way ANOVA and Tukey's post-hoc test using the R 2.14.0 software.

Results: Plasma concentration of insulin was significantly lowered (with 10-15%) already 10 min after applying both doses of butyrate boli, however, plasma GIP level was decreased (with 30-35%) only 30 min after treatment in chickens receiving the higher dose of butyrate, compared to the 0. min values. No significant changes were found regarding the plasma GLP-1 and glucose concentrations after butyrate challenge. Confirming the butyrate-associated changes, no differences were detected concerning any measured parameters in control animals over time.

Conclusion: These results are in contrast with those of mammalian studies, where similarly administered butyrate raised the plasma concentration of both incretins and insulin in mice (3). This can be in association with the species-related significant differences in carbohydrate metabolism of mammals and birds. Our results suggest that the incretins are only partially responsible for the regulation of insulin release in chicken, and also reveal that insulin and incretin homeostasis can be modified by nutritional factors, e.g. by applying butyrate. This study justifies that butyrate has a significant role in regulating the insulin homeostasis of chickens, which is suggested to be partly mediated by incretins. Further investigations on the regulatory mechanisms of insulin release and its influencing by nutrition may provide new possibilities in improving metabolic health and growth performance of chickens.

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86. Effects of NSP degrading enzymes on precaecal nutrient digestiblity and arabinoxylan degradation in turkeys

Einfluss NSP-spaltender Enzyme auf Nährstoffverdaulichkeit und Arabinoxylanabbau bei Puten

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Cereal-based turkey diets are characterized by high amounts of NSP such as the water-soluble arabinoxylan (AX) and *β*-glucans. However, the digestibility of diets containing high levels of NSP is limited but could be improved by enzymes which hydrolyze AX to low molecular compounds and monomer sugars such as xylose and arabinose. The current study aims to investigate the precaecal protein digestibility and enzymatic hydrolysis of AX in the ileal chyme in response to xylanase and ß-glucanase in turkeys.

Methods: 72 one-day old turkeys were randomly allocated to 3 treatments. The control group received a diet without enzymes (T1). Group 2 (T2) was fed a diet with 100 mg/kg diet of a xylanase/ß-glucanase-mixture (ENZY CARBOPLUS; Lohmann Animal Nutrition GmbH, Cuxhaven, Germany, xylanase activity 7000 LXU/g and ß-glucanase activity 600 LGU/g). Group 3 (T3) received 300 mg/kg of the enzyme mixture. The turkeys were kept in pens of 6 animals each and were fed the diets from day 1 to day 28. At day 28, all animals were transferred to individual balance cages and received the experimental diet with added TiO_2 (0.5% of the diet). Excreta were collected for ME calculation over 5 days. At the end of the study, the animals were sacrificed and digesta were collected (1) to determine precaecal protein digestibility and enzymatic hydrolysis of AX.

Results:The analyses of free sugars in the digesta showed a significant increase of arabinose and xylose upon treatment with the enzyme mixture (Table). In particular the high dosage of enzyme mixture was more effective than the low dosage. The enzymatic hydrolysis of AX was accompanied by an improved ileal protein digestibility and ME, which was mainly obvious by comparing the T3 group with the controls (Table). Turkeys that received the high dosage of enzyme mixture showed also an improved weight gain (+9.1%; 80.3 *vs*. 73.6 g/day, T3 *vs*. T1; P=0.009) and FCR (-8.9%; 1.44 *vs*. 1.58 g/day, T3 *vs*. T1; P=0.001) compared to the controls. Data reveal that turkeys that received the enzyme mixture had lower amounts of glucose in the digesta than the controls (Table). We assume that the low glucose content of digesta could have been caused by a higher digestibility of starch and an improved absorption of glucose as it was found in broiler chickens that received xylanase (2).

Table: Free sugars in the digesta, ileal protein digestibility and energy utilization in response to the enzymes in turkey diets. [a,b] Values within a row without common superscripts are different (P£0.05).

Parameter	T1	T2	T3	SEM	P-value
Arabinose (%DM)	0.13[a]	0.17[b]	0.21[b]	0.01	0.003
Xylose (%DM)	0.06[a]	0.10[b]	0.13[c]	0.01	<0.001
Mannose (%DM)	2.01[b]	0.68[a]	1.14[ab]	0.21	0.026
Glucose (%DM)	16.5[b]	4.8[a]	8.5[a]	1.4	<0.001
Galactose (%DM)	0.19	0.19	0.14	0.03	0.655
Ileal protein digestibility (%)	81.2[a]	83.2[ab]	83.7[b]	0.4	0.029
ME (MJ/kg feed)	11.7[a]	11.8[ab]	12.0[b]	0.1	0.004

Conclusion:The results of this study indicate that NSP-degrading enzymes which are added to turkey diets could contribute to increase the performance by improvement of precaecal nutrient digestibility.

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87. Quercetin induces hepatic gamma-glutamyl hydrolase expression in rats by suppressing hepatic miRNA rno-miR-125b-3p

Quercetin induziert die Expression der hepatischen gamma-Glutamylhydrolase über die Suppression der hepatischen miRNA rno-miR-125b-3p

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Exogenous factors such as food components including flavonoids are suspected to influence micro RNA (miRNA) concentrations and thus might exert an impact on molecular targets including enzymes and transporters involved in drug metabolism and excretion. However, aside from promising health promoting activities, one must also bear in mind possible negative effects at least under certain circumstances. Thus, flavonoids have been shown to modulate the expression and activity of enzymes involved in drug and folate metabolism and thus might also interact with chemotherapeutic drugs (e.g. methotrexate) used in cancer treatment. **Methods:** Male Wistar rats (n = 16) were fed either a diet without (C) or with (Q) the addition of 100 ppm quercetin for seven weeks. Body weight gain and liver wet weights were obtained at the end of the experiment. Concentrations of 352 hepatic miRNA were measured using the RT2 miRNA PCR Array System (SABiosciences). Differential expression of miRNAs was assumed with fold changes ≥ 3. Because rno-miR-125b-3p showed the most prominent fold-change (Tab.1), we further analysed the expression of its top predicted target gene (gamma-glutamyl hydrolase) by quantitative real-time PCR. **Results:** Body weight gain and liver wet weights did not differ between groups. Compared to controls 23 miRNAs were differentially expressed in rats fed quercetin (Table 1). In those rats the hepatic miRNA rno-miR-125b-3p was reduced (fold change of -9) and the concentration of the top predicted target mRNA gamma-glutamyl hydrolase was enhanced by a mean factor of 1.8 concomitantly.

Table 1: Selection of differentially expressed hepatic miRNA in quercetin supplemented rats compared to controls after a 7 week feeding period

micro RNA	Fold change	Top predicted target according to TCS	TCS
rno-miR-125b-3p	-9	gamma-glutamyl hydrolase	- 0.61
rno-miR-133b	-7	GA binding protein transcription factor	- 0.73
rno-miR-505	-7	OTU domain containing 4	- 0.8
rno-miR-1	-6	solute carrier family 44, member 1	- 0.59
rno-miR-132	5	high mobility group AT-hook 2	-0.61
rno-miR-125a-3p	5	Endomucin	-0.76
rno-miR-411	4	regulator of G protein signaling 9 binding protein	-0.85
rno-miR-484	3	F-box and leucine-rich repeat protein 18	-0.88

rno = rattus norvegicus; miR: mature micro ribonucleic acid; TCS = Total Context Score (efficiency of prediction; TCS = 0: no efficiency of prediction; TCS ≤ -0,2: sufficient efficiency for prediction; TCS = -1: maximum efficiency of prediction

Conclusions: We conclude from our results, that quercetin induces hepatic gamma-glutamyl hydrolase expression in rats by suppressing hepatic miRNA rno-miR-125b-3p. Because an increased activity of gamma-glutamyl hydrolase was repeatedly associated with resistance to methotrexate, concomitant intake of quercetin with anti-cancer drugs should be monitored carefully.

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88. Does methane emission of dairy cows change with age?

Ändert sich die Methanemission von Milchkühen mit dem Alter?

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The decreasing length of the productive life of dairy cows is cause for concern. Breeding mainly focused on milk yield traits. This results in increasing concentrate proportions in modern dairy cow rations and a decline in fitness traits and, thus, a reduced longevity. Alternative production systems pursuing concentrate reduction and longevity gain interest. However, a high forage proportion will enhance methane emissions per kg of feed ingested and even more so per kg of milk produced as less milk is produced. In addition, the known lower relative methane (CH_4) emissions of growing cattle compared to dairy cows (2) suggests that physiological characteristics are changing with age and CH_4 might further increase with age. Therefore, higher numbers of older cows in herds might render the proportionally lower emissions from the rearing phase useless. In order to clarify this, CH_4 emissions from dairy cows of different age fed either a standard or a zero-concentrate diet were quantified.

Methods: To analyse the development of CH_4 emissions, 2×15 lactating Brown Swiss dairy cows aged between 865 and 3638 days (1st to 7th lactation) were subjected to measurements in respiration chambers. The cows were selected from the two sub-herds of Plantahof, Landquart (CH), which were either fed on a standard diet (forage at *ad libitum* access plus 5 kg/d concentrate) or on a zero-concentrate diet. The forages offered during the experiment were hay, maize silage and grass pellets. Daily average dry matter intake (DMI) and energy-corrected milk yield (ECM) were 20 and 28 kg, respectively. Methane emission, feed intake and milk yield were individually measured in open-circuit respiration chambers at Agrovet-Strickhof (Eschikon, CH) for 2 days after 1 day of adaptation. Gas analysis and data evaluation were performed with equipment from Sable Systems (Las Vegas, USA). Further data was collected subsequently at Plantahof. More details on the experiment can be found in (1). Data were averaged per animal and subjected to regression analysis using the statistical software package R. Diet and age (in days) were included in the models as explanatory variables and ECM and body weight (BW) as covariates.

Results: Mean CH_4 emissions were 21 g/kg DMI, 0.7 g/kg BW and 17 g/kg ECM, and Y_m was 6.4 %. CH_4 emissions were changing ($P<0.05$) with age when related to DMI, BW, ECM and gross energy intake (Y_m). The curve was characterised by an increase from primiparous cows to cows of around 2000 days of age, and a subsequent decline with further increasing age. Diet type did not significantly influence CH_4 emissions, even though numerically higher emissions with the zero concentrate diet were identified. The amount of CH_4 per unit of digestible neutral detergent fibre showed no clear pattern for the tested explanatory variables (Model $R^2=0.09$, Model-P=0.13). Both BW and ECM did not affect methane emissions relative to feed intake traits and were omitted from the regression models, whereas ECM was influencing ($P<0.02$) CH_4/BW and CH_4/ECM.

Conclusion: Age was identified as significant factor for methane emissions of dairy cows, with favourably declining emissions with cow age. Further studies are required to investigate if changing chewing and digestive physiological characteristics, which may influence methane formation, can be associated with these findings. The effect of concentrate in the diet was less distinct than expected. The reason for this might be the use of more highly digestible grass pellets in the zero-concentrate diet and the comparatively low proportion (~20%) of concentrate in the standard diet.

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89. Methane production by avian herbivores

Methanproduktion von herbivoren Vögeln

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Methods: Recent investigations have shown that, in contrast to statements in the literature, adult ostriches (*Struthio camelus*) fed a lucerne-based diet produce similar amounts of methane as similar-sized nonruminant mammalian herbivores (1). Birds from another ratite species, emus (*Dromaius novaehollandiae*), have been claimed to produce no methane; a statement made on basis of measurements in faeces (2). This assumption would fit to the known extremely short digesta retention time in this species (3). Representing a third important ratite species, Rheas (*Rhea* spp.) were found to be intermediate in digesta retention time (3), and hence should show an intermediate methane production. Due to known differences in digesta retention between turkeys (*Meleagris gallopavo*) and geese (*Anser* spp.), with longer retention in the former compared to the latter, similar differences are expected in these species.

Methods: The species comparison comprised six birds each of the species ostrich, emus (2 animals only), rhea, turkey and goose. All animals fed were exclusively on a standard diet of pelleted lucerne offered at *ad libitum* access. This diet was fed for 3 weeks of which the third week was used for the measurements. Measurements included food intake, digesta retention (using both indigestible solute and a particle markers) and faecal excretion for 5-7 days and, subsequently, methane production by open circuit chamber respirometry using sealed wooden boxes complete with portable pumps and gas analysers (Sable Systems, Las Vegas, USA) (1,3). Results were compared among species and with regression lines from nonruminant mammals.

Results: There were significant differences between species in body mass, absolute and relative dry matter intake, digesta mean retention times, and apparent fibre digestibilities. However, absolute methane production per unit of body weight was, for all species, similar to values expected for similar-sized nonruminant mammals. In detail, mean methane production per unit body mass was 0.16, 0.13, 0.21, 0.24 and 0.12 L kg^{-1} d^{-1} in ostriches, rheas, emus, turkey and geese, respectively.

Conclusions: These findings suggest that, with respect to greenhouse gas budgets, herbivorous birds should be treated like similar-sized nonruminant mammals, and that assumptions that these herbivores do not produce methane are not correct. In particular, the results indicate that a link between methane production and digesta retention, as measured by the use of conventional markers, cannot be made, as the three species with longer digesta retention times (ostriches, rheas, turkeys) had, for their body size, a similar methane production as the two species with shorter digesta retention time (emu, geese).

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90. Heat stress adaption of fat metabolism in transition cows

Adaptation des Fettstoffwechsels der Transitkuh im Hitzestress

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In Central Europe, climatic change is expected to cause elevated average temperatures as well as prolonged heat waves. Thus, the zone of thermoneutrality of high-yielding dairy cows of 0-16°C (1) will be exceeded far more often in the future. Ambient temperatures above 16°C or temperature-humidity indexes (THI) of THI > 60 cause serious declines of dry matter intake (DMI) and milk yield (1) challenging future heat stress management even in moderate climates. The aim of the present study was to identify how lipid metabolism in transition cows adapts to high ambient temperatures, as during transition phase, lipid metabolism seriously affects homeorhesis even under thermoneutral conditions.

Methods: German Holstein cows (n=14), dried-off and 3 weeks before 2nd parturition (ap), were assigned to two groups, heat stress (HS) and pair-fed (PF). In experimental period 1 (P1) both groups were kept under thermoneutral conditions (15°C air temperature (AT), THI=60) and fed a total mixed ration ad libitum for seven days. On the following transition day, climatic conditions were adjusted for the subsequent period 2 (P2). During P2, HS cows were kept at 28°C AT (THI=76) and fed ad libitum for another six days while PF cows were pair-fed the same diet but restricted to the extent HS cows reduced their ad-libitum feed intake in P2. The experimental protocol was repeated 3 weeks after parturition (pp). During P1 and P2, blood samples were taken every morning and analysed for non-esterified fatty acids (NEFA). NEFA concentrations were evaluated as area under the curve of six days for P1 (days 2-7) and P2 (days 1-6). On the last day of each period all cows went through a 24-hours indirect calorimetric analysis. At the end of both periods liver biopsies were taken and analysed for mitochondrial (acyl-coA-dehydrogenase very long chain (ACADVL), acetyl-coA-acyltransferase 2 (ACAA2)) and peroxisomal (acyl-coA-oxidase1 (ACOX1), ACAA1) β-oxidation enzymes by qRT-PCR. All variables were examined for differences between HS and PF of the same reproductive stage and for alterations from P1 to P2 using non-parametric exact U-test and Wilcoxon test for paired samples.

Results: In parallel to the decrease of DMI in all groups ($P < 0.05$) fat oxidation per metabolic body weight (FOX/mBW) increased in PF groups from P1 to P2 ($P < 0.05$) but was maintained in HS cows. Temporal increment of heat production per mBW and DMI was numerically lower in HS pp. Increasing plasma NEFA concentrations from P1 to P2 was observed in PF, ap and pp, and HS ap ($P < 0.05$), but not in HS pp. Hepatic expression of mitochondrial enzymes of β-oxidation was unaffected by HS or PF, whereas peroxisomal ACOX1 decreased during P2 exclusively in HS pp ($P < 0.05$) and also ACAA1 tended to decrease in HS pp cows from P1 to P2 ($P = 0.09$).

Conclusions: Unlike PF controls, HS cows did not respond to reduced DMI by increasing FOX/mBW. In early-lactating HS cows, this was paralleled by a blunted lipolytic response. These cows also showed a lower RNA abundance of enzymes involved in peroxisomal but not in mitochondrial β-oxidation. The ATP yield of the β-oxidation in peroxisomes is lower than in mitochondria, and the peroxisomal pathway produces H_2O_2 and metabolic heat from its detoxification. Thus, limiting peroxisomal β-oxidation might be part of a cyto-protective strategy in HS cows by reducing the formation of reactive oxygen species and metabolic heat.

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91. Integrating dynamics of feed intake and metabolic oxidation in late pregnant Holstein cows kept under high ambient temperatures

Zusammenhang zwischen der Dynamik von Futteraufnahme und Nährstoffoxidation bei unter erhöhten Umgebungstemperaturen gehaltenen Milchkühen

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The control of feed intake is a complex process that results from the integration of multiple endogenous and exogenous signals in the brain. Among several endogenous signals, metabolic oxidation has recently been shown to be involved in feed intake control of dairy cows (1). More specifically, short-term feed intake is highly related to periprandial increase of carbohydrate oxidation (COX) and to periprandial decrease in fat oxidation (FOX). Feed intake is strongly reduced when dairy cows are subjected to high ambient temperatures, an issue which is of increasing importance during longer and more frequent heat-waves within the ongoing climate change. However, the understanding of how feed intake is controlled by metabolic oxidation under heat stress conditions is far from clear. Therefore, the aim of the present study was to determine dynamic changes in feeding pattern and metabolic oxidation of late gestation Holstein cows exposed to high ambient temperatures.

Methods: Seven late-pregnant German Holstein cows (3 weeks before 2nd parturition) were kept for 7 d at thermoneutral (TN) conditions (15°C; temperature-humidity-index (THI) =60) followed by a 7 d heat stress (HS) period at 28°C (THI=76). During TN and HS, cows were fed *ad libitum* a total mixed ration (TMR) and kept in tie stall in a climate chamber for 6 d followed by a 24 h-stay in a respiration chamber. During the stay in the respiration chamber, dry matter intake (DMI), oxygen consumption, carbon dioxide and methane production were recorded for 24 h every 6 min (1). Based on the gas exchange data, COX rate and FOX rate were calculated in g/min (1). Differences between TN and HS were analyzed using the Wilcoxon signed rank sum test including the univariate procedure of SAS. Timely association between feed intake and metabolic oxidation were analyzed by cross-correlation functions using Proc Timeseries of SAS providing the time lag between two time series data sets.

Results: As compared to the TN period, cows exposed to HS decreased their DMI by 54%, which was associated with smaller meal sizes (57%), shorter meal duration (77%), and a lower total eating time (79%, each $P < 0.05$). Eating rate expressed as kg DMI per min was also lower under HS than TN conditions (74%; $P < 0.05$). However, daily number of meals (meal frequency) and the mean inter-meal interval were not affected during HS. As a result of the lower feed intake, preprandial COX rate as well as periprandial increase in COX rate were significantly lower in cows exposed to environmental heat (58 and 73%, respectively, $P < 0.05$). Preprandial FOX rate was not affected but periprandial fall of FOX rate was reduced to 38% ($P < 0.05$) when cows were kept under high ambient temperature. Cross-correlation was significant between single meals and COX and FOX, respectively, during both TS and HS conditions ($P < 0.05$), but the time-lag did not differ between TN and HS.

Conclusions: The reduction of daily feed intake in late pregnant HS cows was achieved through a reduction of meal size, meal duration, eating rate and daily eating time with no change in meal frequency. Because preprandial FOX rate was comparable under TN and HS conditions, late-pregnant cows seem to initiate feed intake in response to reaching a certain FOX rate threshold. Comparable time-lags between meals and metabolic oxidation under both TN and HS condition indicate that fundamental mechanisms controlling short-term feed intake by metabolic oxidation are the same for TN and HS late-pregnant cows.

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92. Calculated requirement of ME and pcD lysine in growing finishing boars

Kalkulierter Bedarf an ME und pcv Lysin von Masteberhybriden

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To prevent the risk of boar odour the castration of piglets is common practice, but associated with avoidable pain. Hence, fattening of boars is one viable alternative to improve animal-welfare and to enhance economic aspects, as boars evolve a decreased feed intake and a more efficient feed conversion ratio in comparison to barrows. In addition, the carcass of boars seems to have both lower fat and higher lean-meat content (1). Therefore, the aim of this study was to calculate the ME and precaecal digestible (pcD) lysine requirement of boars based on the overall results of joint research project.

Methods: In general, the estimation of the requirement of boars was based on the assumptions that live weight (LW), LW gain (LWG) and chemical composition of LWG are the primary determinants which are linked to the gross requirement via respective conversion factors (GfE 2008). The latter were assumed to apply for boars as well, while LWG and chemical body composition were experimentally determined for boars (2) and finally modelled by using the Gompertz-equation: $y = a \cdot \exp - b \cdot \exp - c \cdot t$ where y = LW (kg), t = time (d) and a (asymptotic LW), b and c are regression coefficients.

For determination of chemical body composition boar subsets of the growth experiments (3) were used and analysed for crude nutrients according to the methods of the VDLUFA.

Based on the modelled LWG and the progression of chemical composition in LWG the requirements were estimated using the equations by the GfE (4).

Results: The estimated initial, and the asymptotic (adult) LW amounted to ~26 kg (t=0) and 219 kg (a), respectively. The initial and final body protein contents of 159 g/kg LW and 166 g/kg LW corresponded to a LW range between 29 and 121 kg which was used for linear regression: body protein content (g/kg LW) = 157.7 + 0.0673–LW (kg). Body fat content was similarly evaluated (initial and final fat contents of 82 g/kg LW and 205 g/kg, respectively): body fat content (g/kg LW) = 51.98 + 1.2168–BW (kg). Based on these regressions the net requirements were modelled as a precondition for estimation of gross requirements according to GfE (4) (table).

LW	25- 30 kg	30- 35 kg	35- 40 kg	40- 45 kg	45- 50 kg	50- 55 kg	55- 60 kg
LWG (g/d)	733	801	855	903	941	973	955
pcD lys (g/d)	13.8	15.1	16.2	17.2	18.0	18.7	19.2
ME (g/d)	15.65	16.81	18.44	20.01	21.42	22.75	23.92
LW	60- 65 kg	65- 70 kg	70- 75 kg	75- 80 kg	80- 85 kg	85- 90 kg	90- 95 kg
LWG (g/d)	1005	1018	1029	1035	1037	1036	1031
pcD lys (g/d)	19.4	19.8	20.0	20.2	20.4	20.4	20.4
ME (g/d)	24.90	25.90	26.85	27.70	28.47	29.16	29.75
LW	95- 100 kg	100- 105 kg	105- 110 kg	110- 115 kg	115- 120 kg	120- 125 kg	
LWG (g/d)	1023	1008	991	970	947	919	
pcD lys (g/d)	20.4	20.2	19.9	19.6	19.3	18.8	
ME (g/d)	30.27	30.77	31.35	31.84	32.27	32.58	

The (total) ME and pcD lysine requirements were 2689 MJ (29.84 MJ/kg LW accretion) and 1956 g (21.7 g/ kg LW accretion). While the calculated pcD lysine requirement agrees more or less with the recommendations of GfE (4) for barrows and gilts, the ME requirement of boars seemed to be markedly reduced which may result from a constant protein, but a parallel significantly reduced fat accretion.

Conclusion: The results demonstrate that growing finishing boars have only a little higher pcD lysine requirement compared to barrows and gilts whilst the decreased demand for ME might be due to the reduced body fat content. The findings present above are a result of a joint research project "Feeding of boars" (313-06.01-28-1-38.026-10 up to 313-06.01-28-1-38.031-10 BMEL) were fundamentals of recommendations for boar nutrition should be established in cooperation with several research institutes and economy partners.

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93. Impacts of parturition, energy supply and lactation number on glucose uptake of bovine monocyte subsets

Einflüsse der Geburt, der Energieversorgung und der Laktationsnummer auf die Glucoseaufnahme boviner Monozytensubpopulationen

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The peripartal period of dairy cows is characterized by substantial metabolic changes. With the onset of lactation energy requirements for milk production and maintenance exceed feed energy intake and lead to a negative energy balance. The negative energy balance is often associated with peripartal immune suppression and higher incidences of infectious diseases, e.g. mastitis or metritis (1). Due to the high glucose utilization in the udder decreased overall glucose availability may cause energy deficiency and impaired function of immune cells. Monocytes are part of the early inflammatory response and link innate and adaptive immune response. They use primarily glucose as an energy source. Bovine monocytes consist of three subsets: classical monocytes (cM, 89%), intermediate monocytes (intM, 4.4%) and non-classical monocytes (ncM, 5.7%) (2). Therefore, the aim of this study was to evaluate whether glucose uptake of the bovine monocyte subsets is altered by peripartal energy supply.

Methods: Blood samples were collected from 27 German Holstein cows which were included in a feeding trial using a model to induce subclinical ketosis (3). Briefly, cows were allocated to two groups according to their Body Condition Score (BCS). Prior to parturition, BCS low cows received a diet of 80% roughage (50% grass silage and 50% corn silage based on dry matter content) and 20% concentrate. BCS high cows received 40% roughage and 60% concentrate. After calving, the concentrate proportion was raised from 30% to 50% within 2 weeks for the BCS low group and within 3 weeks for the BCS high group, to promote negative energy balance in the BCS high group. Blood was collected at days -42, -14, +7, +21 and +56 relative to parturition. Leukocytes were incubated with the fluorescent probe 2-NBDG for glucose uptake measurement. Glucose uptake of total monocytes and of CD14high monocytes (cM and intM) and CD14low monocytes (ncM) was assessed by flow cytometry. Data were checked for Gaussian distribution and analyzed using one-way or two-way repeated measurements ANOVA.

Results: In a preliminary test cM and intM showed significantly higher *in vitro* glucose uptake compared to ncM ($n = 6$, $P < 0.001$). During the peripartal period *ex vivo* monocyte glucose uptake was not influenced by BCS. Monocyte glucose uptake decreased after parturition and reached a minimum at day +21 ($P = 0.0145$). Glucose uptake of CD14high monocytes decreased at days +7 and +21 ($P = 0.0236$), whereas glucose uptake of CD14low monocytes remained high until day +7 and declined at days +21 and +56 ($P < 0.001$). Glucose uptake did not differ between cows suffering from clinical mastitis and/or metritis and clinically healthy cows. Postpartal, cows with three and more lactations showed lower glucose uptake capacities of CD14high and CD14low monocytes compared to cows in their second lactation ($P = 0.0514$, $P = 0.033$). Blood glucose concentrations declined after parturition ($P < 0.001$).

Conclusion: Neither glucose uptake into monocytes differed between the BCS groups, nor between healthy cows and cows with clinical mastitis and/or metritis. Therefore, there is no evidence that glucose uptake was directly related to disease susceptibility. However, the postpartal decline in monocyte glucose uptake could be based on lower blood glucose concentrations.

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94. Responses of metabolism and performance to repeated short-term feed-restrictions and LPS induced systemic inflammations in dairy cows

Reaktionen des Stoffwechsels und Leistung auf wiederholte kurzzeitige Fütterungsrestriktionen und eine LPS-induzierte systemische Entzündung bei Milchkühen

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Nutrient partitioning towards prioritized tissues during stages of insufficient energy and nutrient supply in dairy cows declines with time after parturition. A negative energy balance (NEB) may occur later in lactation when feed supply and/or quality are insufficient. We investigated if responses of metabolism, performance and immune system to energy deficiency differ between lactational stages. The hypothesis tested was that short-term feed-restrictions have lower effects on metabolism and performance with proceeding lactation. The LPS-induced systemic inflammation was thought to have greater effects in feed-restricted compared to control cows.

Methods: Fourteen pluriparous Holstein dairy cows were grouped according to their previous lactation yield in two groups: a control (CON) group and a restricted (RES) group. The trial lasted from week 3 ante partum (ap) until week 12 post partum (pp). Cows (CON and RES) were fed with grass ad libitum plus additional concentrate throughout the study, except the RES group receiving only grass during 1-week feed-restrictions in weeks 2, 5, 8, and 11 pp. At the end of the first restriction period, LPS from E. coli was infused intravenously (0.5 µg/kg BW) to induce an inflammatory status in CON and RES. DMI and milk yield were recorded daily. Blood was obtained weekly throughout the study, daily during the restriction periods in weeks 2, 5, 8, and 11 pp, and every 0.5 h during the day of systemic LPS challenge. Blood samples were analyzed for glucose, NEFA, BHBA and IGF-1 concentrations. Data were analyzed using a mixed model including group and week as fixed effects in SAS (Version 9.4, SAS Institute, Cary, NC, USA). Significant effects were assumed at a level of $P < 0.05$.

Results: During restriction periods, RES had an elevated grass DMI (0.3-4.2 kg/d) compared to CON ($P < 0.05$). In-between restriction periods, total DMI did not differ between RES and CON ($P = 0.83$). Milk yield was lower for RES in weeks 2, 5, 8 and 11 pp (ca. 5 kg/d) compared to CON ($P < 0.05$)and recovered between restriction periods. On the day of LPS challenge, milk yield in RES dropped more distinct than in CON (9.5 vs. 11.3 kg/d, $P < 0.01$). CON cows recovered faster in milk yield after the LPS challenge. During week 2 pp, plasma concentrations of glucose, NEFA, BHBA were not different between RES and CON, while IGF-1 was lower in RES (41.1 vs. 82.6 ng/mL, $P < 0.01$). During the restriction periods in weeks 5, 8 and 11, NEFA and BHBA concentrations were elevated in RES (up to 0.67 mmol/L NEFA and 0.74 mmol/L BHBA), while glucose and IGF-1 concentration were lower in RES compared to CON (3.77 vs. 4.03 mmol/L glucose, 68.8 vs. 98.4 ng/mL IGF-1 in week 8, $P < 0.05$).

Conclusions: Performance, metabolic and endocrine changes due to feed-restrictions became less with progress of lactation. An induced inflammatory status during early lactation had continued effects on milk yield and DMI in dairy cows.

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95. Metabolic load and dairy cow health-related parameters in herbage based feeding systems

Stoffwechselbelastung und Tierwohl bei Milchkühen in Gras-dominierten Fütterungssystemen

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In Switzerland, herbage feeding with only little input of concentrates plays an important role in milk production. The objective here was to investigate the effects of a solely herbage based diet on production, metabolic, endocrine and health-related parameters in dairy cows.

Methods: Twenty-five multiparous Holstein dairy cows were divided into two groups according to their previous lactation yield (4679-10808 kg): a control (C, n=13) and a treatment group (nC, n=12) from week 3 *ante partum* until week 8 *post partum* (pp). Within C and nC, the median of the preceding lactation yields (7752 kg) was used to split cows into a high (CH, nCH) and low yielding (CL, nCL) subgroup. While CH/CL received fresh cut herbage plus additional concentrate according to their estimated energy and nutrient requirements, no concentrate was fed to nCH/nCL throughout the experiment. Milk yield and DMI were recorded daily. Blood samples were taken weekly and analyzed for IGF-1, glucose, NEFA, BHBA, and health-related parameters haptoglobin (Hp), serum amyloid A (SAA), beta-endorphin (BE) and alkaline phosphatase (AP). Saliva samples were taken bi-weekly and analyzed for cortisol. Data were analyzed using MIXED models in SAS (Version 9.2, SAS Institute, Cary, NC, USA) including week, group and the week × group interaction as fixed effects and the individual cow as repeated subject. Differences between groups over time were detected by the Bonferroni t-test at a level of $P < 0.05$.

Results: Throughout the study, CH had a higher milk yield (35.9 kg/d) compared to the other subgroups (27.2-31.7 kg/d, $P < 0.05$). Plasma glucose (3.51 vs. 3.72 mmol/L) and IGF-1 (66.0 vs. 78.9 ng/mL) concentrations were lower in nCH/nCL compared to CH/CL cows ($P < 0.05$). Plasma NEFA and BHBA concentrations were higher in nCH (1.1 and 1.6 mmol/L) compared to the other subgroups (0.5 and 0.6 mmol/L, $P < 0.05$). Saliva cortisol (0.60 vs. 0.68 ng/mL), SAA (0.60 vs. 0.87 mcrg/mL), Hp (728 vs. 909 U/L), BE (30.0 vs. 32.1 pg/mL) and AP (48.5 vs 45.9 mg/mL) were not different among C and nC.

Conclusions: In conclusion, in herbage dominated feeding systems without supplementary concentrate especially high yielding dairy cows experience a higher metabolic load during early lactation leading in turn to a reduced lactational performance compared to cows of a similar potential fed according to their needs. Low yielding dairy cows can perform well without concentrate supplementation. Interestingly, the commonly accepted welfare-related parameters cortisol, Hp, SAA, BE and AP did not indicate a reduced animal welfare induced by metabolic stress.

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96. Insulin signaling in adipose tissues of periparturient dairy cows under the influence of an energy-dense diet and nicotinic acid supplementation

Insulinsignaltransduktion in Fettgeweben von peripartalen Milchkühen unter dem Einfluss einer energiereichen Diät und Nikotinsäuresupplementierung

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Question. The main physiological effect of insulin on adipose tissue is to promote glucose uptake and lipogenesis and to suppress lipolysis. The tensed metabolic condition of transition cows, which develops due to energy deficit at the onset of lactation, is known to be associated with a reduced insulin sensitivity of adipose tissue in support of energy mobilization and glucose shifting to the mammary gland. However, understanding of the exact cellular mechanisms to achieve this reduced insulin action is still incomplete. Therefore, this study aimed to describe the changes of expression and activation of signaling proteins involved in insulin transduction in subcutaneous and abdominal adipose tissues of dairy cows around parturition. Targeted dietary interventions, including different energy densities and supplementation of nicotinic acid, were used to influence postpartum energy metabolism.

Methods. Twenty German Holstein cows were fed an energy-dense (60% concentrate; 7.63 MJ NEL/kg DM) or an energetically adequate (30% concentrate; 6.97 MJ NEL/kg DM) diet from 42 days prepartum until parturition to induce a greater or a lower rate of postpartum lipid mobilization, respectively [1]. In both groups, cows received 0 (n=10) or 24 g/day per animal (n=10) dietary nicotinic acid, an antilipolytic agent, to further enhance differences in lipid mobilization [2]. Subcutaneous (SCAT) and retroperitoneal adipose tissue (RPAT) biopsy samples were collected at 42 days prepartum and at 1, 21, and 100 days postpartum. Protein expression and/or phosphorylation of insulin receptor and further key components of the insulin signaling pathway were detected by Western blotting (results are shown as arbitrary units, all data are shown as mean±SEM). Expression data were analyzed for effects of periparturient time, diet, and adipose tissue localization by ANOVA with Tukey's post-test.

Results. In the prepartum period, cows with an energy-dense diet had greater DMI than cows with an energetically adequate diet (16.74±0.39 kg/d vs. 14.29±0.25 kg/d, respectively; $P<0.01$). However, prepartum dietary manipulations did not significantly affect postpartum DMI (19.50±0.25 kg/d, groups pooled together), nor milk yield (41.38±0.34 kg/d, groups pooled together), nor protein expression or phosphorylation. The expression of insulin receptor was lower ($P<0.001$) at 1 day postpartum (SCAT: 0.38±0.07, RPAT: 0.40±0.07) than prepartum (SCAT: 2.79±0.39, RPAT: 2.48±0.45). At 21 days postpartum (SCAT: 1.19±0.12, RPAT: 1.49±0.16) and 100 days postpartum (SCAT: 1.29±0.15, RPAT: 1.69±0.10) the receptor expression was still decreased compared to the prepartum level ($P<0.001$). Consequently, most of the studied downstream signaling proteins also had a reduced expression or phosphorylation in the early postpartum period (time effect for each protein: $P<0.001$).

Conclusions. The present data indicate that adipose tissue had a decreased capacity to respond to insulin during the early postpartum period, irrespectively of dietary energy intake and nicotinic acid supplementation. This can be identified as a physiological control mechanism to promote adipose mobilization and glucose shifting, in order to overcome energy deficit and to support milk production.

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97. Relationship between circulating leptin concentrations and adipocyte mitochondria in nonlactating dairy cows during a course of overcondition

Zusammenhang zwischen zirkulierenden Leptinkonzentrationen und Mitochondrien in Fettzellen bei nichtlaktierenden Milchkühen im Verlauf einer Überkonditionierung

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Mainly produced in adipose tissue (AT), leptin is involved in the regulation of energy metabolism. In dairy cows, leptin is upregulated during late lactation and decreased before parturition, when body reserves are re-filled to prepare the animal for the subsequent lactation. The number of mitochondria, the main cellular energy suppliers, increased in AT during fattening in nonlactating dairy cows (1). Based on the metabolic effects leptin exerts in AT, we hypothesized that circulating leptin serum concentrations are related to the number of mitochondria in bovine AT. We aimed to investigate the association between circulating leptin concentrations and the mitochondrial (mt) DNA copy number, reflecting the number of mitochondria within a cell, in relation to adipocyte sizes from dairy cows with increasing body condition. In addition, programmed cell death (apoptosis), which is negatively correlated to adipocyte size in bovine, has been determined.

Methods: Eight nonpregnant, nonlactating German Holstein cows (age: 4 - 6 years) were moved from a straw diet to a high energy diet by stepwise increasing the concentrate and silage portions from 0 to 60 % and 0 to 40 % of DM within 6 weeks (wk). This diet was maintained for further 9 wk. The mean body weight (BW) gain per cow was 243 ± 33.3 kg and body condition score (BCS) increased from 2.3 ± 0.4 to 4.5 ± 0.4 throughout the experiment ($P = 0.001$). Blood samples were collected each month and serum leptin concentrations were quantified by ELISA. Subcutaneous AT from the tailhead region was biopsied at the onset, after 8, and 15 wk of the experiment. The samples were either snap frozen in liquid nitrogen for isolating genomic DNA or were fixed in 4% formaldehyde for subsequent paraffin embedding and evaluation of adipocyte sizes (μm^2) as well as for the analysis of apoptosis by a TUNEL assay. The relative mtDNA copy number/cell was quantified by a multiplex qPCR targeting the 12S rRNA gene using the ß-globin as an endogenous nuclear control gene. Calculation of mtDNA copies/cell was described earlier (2). Data were analyzed using linear mixed models and associations between parameters were assessed by the Spearman correlation (SPSS).

Results: Adipocytes tended to be enlarged ($P = 0.090$) and both mtDNA copy numbers and leptin concentrations increased 4.7-fold and 2.2-fold, respectively, until wk 8 of the trial. Moreover, the portion of apoptotic cells decreased 2.5-fold ($P = 0.026$) within the first 8 wks of the trial. Thereafter, all variables investigated herein were not further increased at the end of the trial. Positive correlations were observed between mtDNA copies/cell and adipocyte sizes ($r = 0.388$, $P = 0.067$), leptin ($r = 0.707$, $P < 0.001$), BW ($r = 0.596$, $P = 0.003$) and BCS ($r = 0.503$, $P = 0.012$).

Conclusions: Increased mtDNA content in enlarged adipocyte sizes may sustain or even increase the energy supply, thus the portion of apoptotic cells was decreased. Leptin might inhibit lipid accumulation (3) and increasing leptin concentrations may thus inhibit a further enlargement of the adipocytes, leading to stagnating mtDNA copies/cells. Stable adipocyte sizes on the other hand will lead to stagnating leptin concentrations.

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98. Fractional gluconeogenesis estimate in sheep measured by deuterium oxide method does not differ between intravenous and intraruminal administration routes of D_2O

Die Messung der fraktionellen Gluconeogenese beim Schaf unter Verwendung der Deuteriumoxid-Methode unterscheidet sich nicht zwischen intravenöser und intraruminaler D2O-Applikation

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The D_2O method has been used to measure fractional gluconeogenesis (GNG) in humans. The advantage of this method is that all contributions of gluconeogenic substrates are considered (1). In ruminants, the main precursor for de novo GNG is rumenal propionate which fundamentally differs from gluconeogenic precursors of monogastrics. We aimed to determine whether the route of D_2O administration affects equilibration of deuterium with protons from water in various body water pools, and whether this results in differences of deuterium enrichment of propionate and estimates of fractional GNG.

Materials and Methods: Four sheep (23.5 ± 1 kg BW), equipped with a rumen fistula and a jugular vein catheter, were fed a pelleted ration (35 g/kg BW and d; 9 MJ ME/d) at 2-h intervals. Water was offered ad lib. To label body water, sheep were given two boli of 7 g D_2O (99.2 atom% (AP) D/kg BW) at 0800 and 1200 h either into the rumen (IR) or into the jugular vein (IV) in a balanced cross-over design. Two weeks separated each site of administration. Plasma was sampled prior to and hourly for 11 h following the first bolus whereas rumen fluid and urine were collected prior to and at 3, 6, 9 and 11 h. Samples were diluted and D_2O enrichments were measured by isotope ratio mass spectrometry. Deuterium labeling of rumenal propionate and plasma glucose was measured by GC-MS (2). Fractional GNG was calculated from the ratio of D enrichment at C-5 of glucose (labeling via GNG only) to that at C-2 (labeling during GNG and glycogenolysis). Data were analyzed with repeated measures ANOVA using PROC MIXED of SAS with the main fixed factors route of administration, order of routes, time, and animal as random. Data is presented as means ± standard error.

Results: A plasma D enrichment plateau was reached 2 h after the first bolus (IR: 0.69; IV: 0.71 AP excess (APE); $P>0.1$) with a further increase to a second plateau at 1400 h but no difference among the routes (IR: 1.46; IV: 1.48 APE; $P>0.1$). No difference was observed in urine D enrichment between the two routes of administration ($P=0.408$) at any time ($P>0.05$). Rumen fluid D enrichment attained a plateau 6 h after the first bolus (IR: 1.51; IV: 1.43 APE; $P>0.1$). The D enrichment of propionate in rumen fluid as a precursor for GNG showed differences in enrichment kinetics with faster labeling with the IR route (3h: $P<0.10$; 6h: $P<0.05$). GNG estimates derived from IR and IV routes did not differ ($P=0.833$) which resulted in an overall GNG estimate of 58.8%.

Conclusions: For measurement of fractional GNG using the D_2O method, the D labelling of body water pools was similar with the IR and IV routes. Both routes of administration yield similar estimates of GNG in ruminants.

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99. Indicator substances in breath samples of early lactation dairy cows for detection of subclinical ketosis

Indikatorsubstanzen in Atemproben von Milchkühen zur Detektion von subklinischen Ketosen in der Frühlaktation

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The energy deficit of dairy cows in early lactation entails mobilisation of body reserves to supply energy. The excess of body fat mobilisation results in the formation of ketone bodies and their excretion as acetone in breath. The present study was conducted to identify substances in breath of dairy cows which could serve as indicators for detection of subclinical ketosis (sk) in early lactation.

Methods: Ten pluriparous Holstein cows were fed according to an animal model to induce sk. In brief, four cows with a low body condition score (BCS) (LC group) and six cows with high BCS (HC group) received six weeks ante partum (a.p.) a diet consisting of 20% or 60% concentrate (on dry matter basis), respectively. The mean BCS at 14 days a.p. of the LC group was 2.81 ± 0.12 and of the HC group 3.79 ± 0.23. After parturition the concentrate proportion in the diet was 30% and increased within 14 d for the LC cows and within 21 d for the HC cows up to a level of 50% concentrate. Blood and breath samples (immediately after blood sampling) were taken on d 14 a.p., 7, 21 and 56 d post partum. Blood samples were analysed for serum concentrations of β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). The breath samples were analyzed by gas chromatography for acetone, dimethylsulfid (DMS) and ethylmehtylketone (EMK). Data were analysed by using the MIXED and CORR procedure of SAS 9.2. Values represent LS-means ± SEM, unless otherwise specified.

Results: The BCS for LC cows (2.78 ± 0.07) was lower (P = 0.009) than for HC cows (3.08 ± 0.08). Concentrations of NEFA in blood serum were 0.32 mmol/L±0.04 for the LC group and 0.54 mmol/L ±0.03 for the HC group and differed significantly (P=0.002). The BHB concentration in blood serum and the acetone concentration in breath showed similar development within the groups (Figure 1).

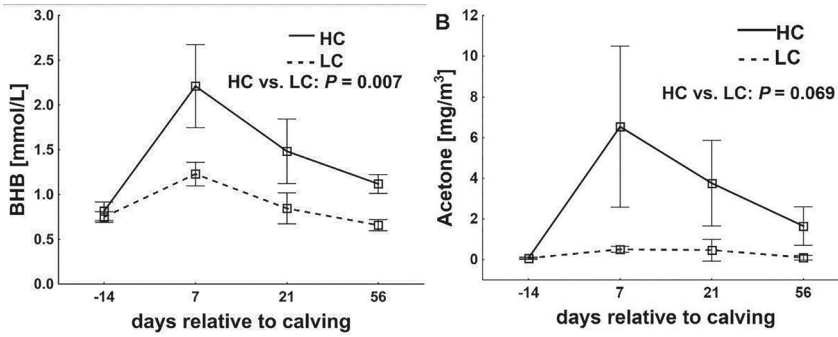

Figure 1. BHB concentration in blood (A) and acetone concentration in breath (B) (means ±SEM).

No differences were observed for DMS (P=0.101) and EMK (P=0.205) concentration in breath between the groups. Correlation coefficients of the blood and breath variables are shown in Table 1.

Table 1. Pearson correlation coefficients between NEFA and BHB with possible indicator substances for subclinical ketosis in breath (acetone, dimethylsulfid and ethylmethylketone)

	Acetone	Dimethylsulfid	Ethylmethylketone
NEFA	0.767***	0.377*	0.113
BHB	0.638***	0.192	0.087

*P<0.05; **P < 0.01; ***P < 0.001

Conclusion: Among the tested substances in breath acetone showed closest correlations with NEFA and BHB in blood serum and may therefore be used as an indicator for sk.

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100. Effects of intensive milk feeding in calves on intestinal growth, immune status and hepatic energy metabolism

Einfluss einer intensiven Milchfütterung beim Kalb auf Darmwachstum, Immunstatus und Energiestoffwechsel der Leber

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Feeding unlimited amounts of milk in the early postnatal period was recently shown to promote body growth in calves without impairing concentrate intake and pre-weaning rumen development (1). In addition, plasma concentrations of glucose and insulin as well as maturation of the somatotropic axis were enhanced during intensive milk replacer (MR) feeding (1). In the present study, we hypothesised that intensive milk feeding affects endocrine regulation of hepatic energy metabolism, immune status and intestinal growth before weaning.

Methods: Holstein and Holstein × Charolais calves were fed colostrum (5 l/d) for first 3 d of life and then different amounts of MR (125 g/l water) by automate feeder (6 l/d for 8 wk (RES; n=14) or unlimited until d 35 (ADLIB; n=14). For ADLIB MR intake was reduced during wk 6 stepwise to 6 l/d and then kept constant. Concentrate and hay were freely available. Blood samples were taken on d 1, 2 and 7, then weekly until wk 8 of life for determination of plasma concentrations of immunoglobulin (Ig) G_1, G_2, M, fibrinogen and haptoglobin (2). Calves were slaughtered before weaning at d 60 ± 2 and small intestine and liver were removed. Mucosa samples from duodenum, proximal, middle and distal jejunum and ileum were taken for measurement of villus height (VH) and crypt depth (CD). In liver, glycogen concentration and mRNA abundance of insulin-like growth factor (IGF)-I, IGF and insulin receptor, IGF binding proteins 2, 3 and 4, glucose-6-phosphatase, pyruvate carboxylase, phosphoenolpyruvate carboxykinase (cytosolic isoform; *PCK1*) and glucose transporter GLUT 2 was measured by qRT-PCR. Data were analysed by Mixed Model of SAS with feeding and time or gut segment as fixed effects.

Results: Plasma concentrations of IgG_1, IgG_2 and IgM increased ($P < 0.05$) after first colostrum intake; IgG_1 was highest on d 2, then decreased ($P < 0.05$), IgG_2 ($P < 0.05$) decreased from d 2 to d 7 remaining constant thereafter, and IgM showed peak concentrations on d 2, 14 and 56. Plasma concentrations of fibrinogen ($P < 0.05$) increased (ADLIB until d 14, RES until d 21) and tended to be greater ($P < 0.1$) in RES, whereas haptoglobin were unchanged by time or feeding. Hepatic glycogen concentration was unaffected by feeding as was mRNA abundance of genes related to the somatotropic axis. Only mRNA abundance of *PCK1* was greater ($P < 0.05$) in ADLIB than in RES. Morphometric measurements in small intestine indicated greatest VH and smallest CD in middle jejunum ($P < 0.001$) with feeding × gut segment interactions ($P < 0.001$) for VH, CD and VH/CD ratio and a trend for greater VH and VH/CD in ADLIB than in RES.

Conclusions: Intensive milk feeding did not affect calves' immunological status, probably because colostrum intake was equal between groups. Differences in plasma fibrinogen may point to a feeding effect on hepatic acute phase protein response. The hepatic somatotropic axis was not affected at slaughter, although associated differences in blood plasma were seen during the intensive milk feeding period (1). Intensive milk feeding may influence gluconeogenesis in liver indicated by elevated mRNA abundance of *PCK1* in ADLIB. Concerning intestinal growth, results may imply slight stimulation of villus growth with intensive milk feeding.

(1)HAMMON, H.M. et al. (2014) Proc. Nutr. Physiol. 23: 64.
(2) Furman-Fratczak, K.et al. (2011) J. Dairy Sci. 94: 5536-5543.

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101. Estimation of total body fat mass by bioelectrical impedance analysis and deuterium-oxid-dilution method in cows - a validation study

Bestimmung der Körperfettmasse mittels Bioelektrischer Impedanz-Analyse und Deuteriumoxid-Verteilung bei der Kuh – eine Validierungsstudie

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Body fatness and degree of body fat mobilisation around parturition strongly influences feed intake, energy partitioning and metabolic adaptation during late gestation and early lactation in dairy cows. Estimation of body fatness by measurement of back fat thickness (BFT) or body condition scoring seems to be not meaningful enough to predict individual body fat mass and mobilisation (1). Therefore, the aim of the study was to validate bioelectrical impedance analysis (BIA) as a method to predict total body fat in cows and to compare BIA measurements with the deuterium-oxide (D_2O) dilution method for estimating total body fat in cows.

Methods: Body weight (BW) and BFT were determined in 18 Charolais x German Holstein (HF) crossbred and 2 HF cows. On 32 ± 2 days in milk (DIM) total fat mass (tFM) was measured by BIA and D_2O dilution method. For the latter a bolus of 0.23 g/kg BW D_2O (60 atom %, AP) was injected i.v. and plasma D enrichment (AP excess, APE) was analysed 4 h post injection, (APEp4h). Cows were slaughtered on 36 DIM and dissected for measurement of tFM *ex vivo*. On day 4 prior to slaughter BIA was performed using a four-electrode interface and time domain-based measurement system (IMPSPEC, Meodat GmbH, Ilmenau) consisting of a voltage/current converter for applying current stimulus (100µA RMS, multisine signal, frequency range 1.4 kHz - 1 MHz) and an amplifier for monitoring voltage across the sensor electrodes. Measurements were carried out with 5 different electrode configurations (V1-V5). The BIA data (resistance extra cellular space, resistance intracellular space, cell membrane capacity, phase angle) were extracted differently: first by least square fit, gaining fat mass (FM), cell volume (CV), and total body water (TBW), secondly by Cole-Cole Model gaining FM_k, CV_k and TBW_k that were used for regression analyses. TBW_D content was calculated as D_2O dosage/APEp4h*100/1000. Regression analysis for the dependent variable tFM was performed with the multiple linear PROC REG procedure of SAS. Pearson correlations were calculated to determine relations between data with the PROC CORR procedure of SAS.

Results: Cows varied widely in tFM *ex vivo* (tFM = 165.08 ± 63.61 kg; mean ± SD). Calculated TBW_D (58.5 ± 4.9 %) was negatively related to tFM (r = -0.83). Calculated relative FM from BIA was positively related to tFM ($P < 0.05$; r = 0.79) and TBW and CV from BIA were negatively related to tFM ($P < 0.05$; r = -0.66). Higher TBW and higher CV represent lower tFM. Prediction of tFM by D enrichment and BIA are shown in the Table:

Method	tFM =	R^2
BIA V1	$-460+1136*EI+10192*CV-30605*TBW_k+30555*CV_k+0.17*FM_k$	0.99
BIA V2	$-791+892*EI+2042*TBW-7386*TBW_k+6981*CV_k+0.17*FM_k$	0.86
BIA V3	$542-788*TBW-34*FM-5237*CV_k+34.5*FM_k$	0.99
BIA V4	$-153.2+0.41*FM$	0.79
BIA V5	$157-509*EI+22724*CV+22*FM-22512*CV_k-21*FM_k$	0.99
D_2O plasma	$-298+11642*APEp4h+0.18*BW+14.8*BFT$	0.90

EI=Electrode interval, CV=Cell volume, TBW=total body water, FM=fat mass, k= data fitted by Cole-Cole Model, APEp4h=atom percent excess plasma 4h, BW=body weight, BFT=back fat thickness

Conclusion: Prediction of FM by BIA and D_2O dilution was closely related to estimated tFM obtained by dissection. Both BIA and D_2O dilution result in similarly good predictions of body fatness in adult cows, thus BIA can be used as non-invasive and cost-efficient method to predict tFM.

1) WEBER et al., (2013): J. Dairy Sci. 96: 165-180

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102. Responses to an energy deficiency in early and mid-lactation and its implication to robustness of dairy cows

Reaktionen auf ein Energiedefizit in Frühlaktation und Mitte der Laktation und deren Bedeutung für die Robustheit von Milchkühen

*Gross J., Bruckmaier R. – Bern

Nutrient partitioning towards prioritized tissues during stages of insufficient energy and nutrient supply is a strategy to maintain homeostasis and achieve homeorhesis in dairy cows. Despite similar metabolic loads, the adaptive responses vary tremendously between animals.

The aim of this study was to investigate if cows respond in a repeatable manner to a negative energy balance (NEB) at different stages of lactation. Twenty-five dairy cows experienced a NEB after parturition and a second NEB for 3 wks induced by feed-restriction in mid-lactation.

Methods: Twenty-five multiparous Holstein dairy cows (3.0 ± 1.1 parities; mean ± SD) were studied during a period from parturition up to approximately 120 days in milk (DIM). Cows experienced a NEB during the first weeks of lactation, and after getting into a positive EB, all cows were exposed to a 3-wk feed-restriction period providing 50% of energy and nutrient requirements starting at around 100 DIM (1). Data on dry matter intake and milk yield were collected daily. Energy balance and energy expenditure for maintenance and lactational requirements were calculated according to the guidelines of the German Society of Nutrition Physiology. Blood samples for analysis of plasma concentrations of glucose, NEFA, BHBA, cholesterol, and IGF-1 were obtained once weekly.

In a retrospective analysis, cows were ranked according to their highest plasma NEFA-concentration in early lactation (independent of time). The cows with the 33% highest and 33% lowest NEFA concentrations (8 animals each) were selected and classified either a high response (HR) or a low response (LR) group, respectively. Data of the two groups were compared using the MIXED model in SAS (Version 9.4, SAS Institute, Cary, NC, USA) including wk, group (HR or LR), parity, and the wk × group interaction as fixed effects and the individual cow as repeated subject. Differences between HR and LR over time were detected by the Bonferroni t-test. Significant effects were assumed at a level of $P < 0.05$.

Results: Before parturition, no differences in the studied variables were detected between LR and HR. After parturition, milk yield and ECM was higher for HR compared to LR ($P < 0.05$). During feed-restriction, no differences in ECM between LR and HR were found. Although plasma concentrations of glucose and cholesterol showed group differences in early lactation, but not during feed-restriction, the plasma concentrations of NEFA, BHBA, and IGF-1 showed a repeatedly similarly directed, but different response to a NEB at the two stages of lactation despite the similar deficiency for both groups. HR had higher NEFA, BHBA and lower IGF-1 concentrations compared to LR at the two stages of lactation ($P < 0.05$).

Conclusions: These similar adaptive responses at different time-points may reflect an underlying genetic background to enable a sufficient and rapid supply of mobilization derived nutrients. Cows responding more intense to an energy deficiency in early lactation repeatedly showed higher plasma concentrations of NEFA and BHBA despite a similar milk production as observed in cows with lower NEFA and BHBA concentrations.

1) GROSS et al. (2011): J. Dairy Sci. 94(4): 1820-1830.

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103. Effects of metabolic and inflammatory challenges on cortisol secretion in dairy cows

Auswirkungen metabolischer und inflammatorischer Challenges auf die Cortisolsekretion bei Milchkühen

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Cortisol in dairy cows is released in an episodic manner underlying a circadian rhythm. Recent studies described contradictory effects of nutrition and metabolism on the secretory pattern of cortisol. However, up to now the direct effects of single metabolites during various metabolic conditions on the glucocorticoid secretion in dairy cows have not been described. The objective of this study was to investigate the effects of long-term (56 h) manipulated metabolic states, i.e. manipulated plasma concentrations of glucose and BHBA, on the release of cortisol in mid-lactating dairy cows. Besides the concentration of cortisol, its pulsatile secretory pattern was studied in combination with an acute immune challenge through an intramammary LPS challenge.

Methods: Twenty-five mid-lactating dairy cows (parity: 3.1 ± 1.2, 28 ± 5 weeks post-partum; mean ± SD) were randomly assigned to one of four treatments. Treatment groups included either a hyperinsulinemic hypoglycemic clamp (HypoG, n = 6), a hyperinsulinemic euglycemic clamp (EuG, n = 6), infusion of β-hydroxybutyrate (HyperB, n = 5) or an infusion with physiological saline solution for the control group (NaCl, n = 8) for 56 h.

Beginning at 48 h of infusion, an intramammary LPS challenge was performed as described previously (1). Blood samples for analysis of cortisol were taken every hour beginning 24 h after the start of infusions. Total cortisol was measured by radioimmunoassay. Intra- and inter-assay CV for cortisol were 8.5 and 9.1%, respectively.

The cortisol profile from 09:00 a.m. to 05:00 p.m. of the second and third day during infusions (without and with intramammary LPS challenge, respectively) were analyzed using the PULSAR program for the evaluation of episodic hormone release. Default cut-off criteria were set at G(1) = 4.40, G(2) = 2.60, G(3) = 1.92, G(4) = 1.46, and G(5) = 1.13 standard deviations of the assay to keep the error rate of falsely identified peaks below 5%. Variables derived from the PULSAR program included the mean of the calculated smoothed baseline, the number of peaks, mean height, length, area and amplitude of peaks, the peak interval, and the area under the curve (AUC) over the calculated baseline (AUCb) and AUC over the 0-line (AUCt).

Data of cortisol concentration and characteristics of its pulsatile release were analyzed by the general linear model (GLM) procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC, USA), including treatment (HypoG, EuG, HyperB or NaCl), and day of infusion (without or with LPS treatment) as fixed effects. Differences between means were localized by Tukey's test. P-values < 0.05 were considered to be significant.

Results: Different metabolic states induced by infusion treatments affected the characteristics of cortisol secretion (elevation of baseline (HypoG, HyperB) and decrease of peak length (HypoG)), while amplitude, peak interval, height, peak area, area under the curve (AUC) above baseline (AUCb) and the total AUC above the 0-line (AUCt) were not different between infusion treatments. The induced inflammatory response due to the intramammary LPS challenge for the last 8 h at simultaneously maintained infusion treatments diminished the pulsatile nature of cortisol release, while AUCb (and AUCt, respectively) was lowest for HypoG compared to HyperB and NaCl.

Conclusions: This study indicates that single metabolites (glucose, BHBA) and their availability or turnover (in case of glucose) have a different impact on the regulation of cortisol secretion resulting in changes of its pulsatile release. Furthermore, cortisol release during intrammammary inflammation was found to be higher in HyperB, EuG and NaCl compared to HypoG. This finding emphasizes the regulatory role of the current metabolic status on the cortisol release during inflammation.

1) VERNAY et al. (2012): J. Dairy Sci. 95: 2540-2549.

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104. Cholesterol metabolism during a negative energy balance in dairy cows at two stages of lactation

Cholesterinstoffwechsel während einer negativen Energiebilanz bei Milchkühen zu zwei Phasen in der Laktation

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The negative energy balance (NEB) in early lactation has considerable effects on the cholesterol metabolism of dairy cows. The objective of this study was to investigate the response of plasma and milk lipids, enzyme activities and hepatic mRNA expression of transcripts encoding for factors involved in cholesterol metabolism to a NEB in early and mid-lactation. We hypothesized that the adaptation of the cholesterol metabolism to a NEB at around 100 DIM is physiologically distinct from responses occurring during the early lactational NEB.

Methods: Fifty multiparous Holstein dairy cows were studied during 2 periods with a NEB: Directly after parturition during the lactational NEB (week 1 postpartum (pp)) and after around 100 DIM (week 14 pp) during a period of feed restriction for 3 weeks. In week 14 pp cows were in a positive energy balance and split into two groups: 25 control (CON) and 25 restricted (RES) cows. Blood and milk lipid concentrations [triglycerides (TG), cholesterol, lipoproteins] and enzyme activities [phospholipid transfer protein (PLTP), lecithin-cholesterol acyltransferase (LCAT)] related to cholesterol homeostasis were analyzed. Hepatic mRNA abundance of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGC) synthase 1 (HMGCS1) and HMGC reductase (HMGCR), sterol regulatory element-binding factor (SREBF) -2, microsomal triglyceride transfer protein (MTTP), ATP-binding cassette transporter (ABC) A1 and ABCG1 were measured. Changes in blood parameters, milk cholesterol and hepatic mRNA abundances between week 1 pp and week 17 pp during feed restriction in RES cows as well as the effect of feed restriction between week 14 to 17 for both groups were evaluated with a MIXED model including group and parity as fixed effects. Furthermore, concentrations of blood parameters, milk cholesterol and hepatic mRNA abundance of week 14 were used as co-variable and the individual cow as repeated subject. Differences between groups were detected by the Bonferroni t-test. P -values < 0.05 were considered to be significant.

Results: While plasma concentrations of TG, cholesterol, VLDL-cholesterol (VLDL-C) and LDL-C were lower for cows in wk 1 pp (0.11 ± 0.00; 1.59 ± 0.05; 0.02 ± 0.00; 0.69 ± 0.02; mmol/L), these concentrations increased in RES cows from wk 14 to 17 pp (TG: 0.13 ± 0.01 to 0.15 ± 0.01; cholesterol: 4.76 ± 0.15 to 5.45 ± 0.18; LDL-C: 2.76 ± 0.11 to 3.27 ± 0.14; mmol/L) compared to CON cows ($P < 0.05$; in week 17: TG: 0.13 ± 0.00; cholesterol: 4.93 ± 0.18; LDL-C: 2.87 ± 0.15; mmol/L). Whereas in wk 1 pp PLTP activity was increased and LCAT activity was lower, activities of PLTP and LCAT did not differ between wk 14 and 17 pp in CON and RES cows. Cholesterol concentration in milk did not change from wk 14 to 17 pp, whereas cholesterol mass in milk was decreased in wk 17 pp for RES cows (1.59 ± 0.11 g) and tended to be lower in RES cows compared to CON cows (1.97 ± 0.10 g; $P = 0.06$). On the contrary, cholesterol concentration and mass in milk were higher in wk 1 pp. SREBF-2, HMGCS1, HMGCR, MTTP, ABCA1 and -G1 showed no changes during the experiment. In contrast, during the NEB at the onset of lactation the expression of HMGCS1, HMGCR, SREBF-2 and ABCA1 were increased.

Conclusions: In conclusion, increased plasma concentrations of TG, cholesterol, VLDL-C, and LDL-C during the feed restriction period suggest that in later stages of lactation the liver is able to enhance the export of generated TG as VLDL. The diminished milk cholesterol mass might represent a measure to save cholesterol for the constitution of VLDL.

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105. Bioavailability of deoxynivalenol (DON) and DON sulfonates 1, 2 and 3 in pigs fed with sodium sulfite treated DON contaminated maize

Die Bioverfügbarkeit von Deoxynivalenol (DON) und den DON-Sulfonaten 1, 2 und 3 nach Aufnahme von Natriumsulfit – behandeltem DON-kontaminiertem Mais beim Schwein

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Due to the high sensitivity of pigs to DON contamination of feed, which results in adverse effects on performance and health counteracting strategies need to be developed. A possibility to use contaminated batches exceeding specified toxin concentrations could be a chemical detoxification. In a previous experiment the significant reduction of the DON concentration in maize could be demonstrated after a wet preservation method with sodium sulfite (1). As a result, the preserved material contained DON sulfonates (DONS 1, 2, 3) whose toxicological properties were tested in vitro (2). The investigation of the toxicokinetics of DON and DONS in pigs allows conclusions about the systemic absorption of the individual compounds.

Methods: The study was carried out with 16 male castrated pigs with a mean body weight of 39 kg, which were housed in balance cages to facilitate an easy handling during the test duration. For a serial blood sampling all animals underwent surgery wherein they were equipped with two permanent intravenous catheters in the external vena jugularis. The following six treatments were tested: DON maize diet with 6 mg DON/kg feed, DON maize diet supplemented with dry sodium sulfite (5 g/kg) and four different wet preserved DON maize diets (in Figure divided in kernels after 37 and 79 days preservation duration as well as meal after 37 and 79 days) treated with sodium sulfite (10 g/kg maize).

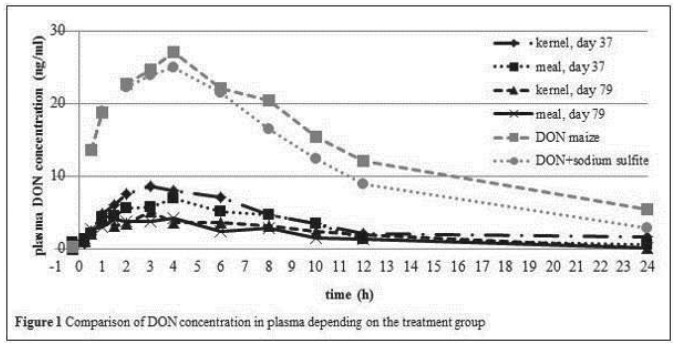

Figure 1 Comparison of DON concentration in plasma depending on the treatment group

Results: Preliminary results of the kinetics of DON are represented in the Figure 1.

Generally, DON was rapidly absorbed and reached maximum plasma concentrations approximately 3 to 4 hours after feeding the experimental diets irrespective of diet type. In contrast, the corresponding maximum plasma concentrations and the areas under the curve (AUC) differed significantly between the untreated DON containing diet and all DON containing diets wetly preserved in the presence of sodium sulfite. Simple addition of sodium sulfite to the DON containing diet without a preservation was ineffective in reducing the AUC. Among the DON containing diets wetly preserved with sodium sulfite a longer preservation time seemed to enhance the effectiveness of the measure. DONSs could not be detected in plasma so far after feeding DON contaminated feed preserved with sodium sulfite and containing DONSs.

Conclusion: Wet preservation of DON contaminated maize with sodium sulfite decreased the bioavailability of DON markedly which, however, could not be explained by increased blood levels of the DONSs which evolve during wet preservation. The reasons for this phenomenon need to be examined further.

(1) Paulick M, Rempe I, Kersten S, Schatzmayr D, Schwartz-Zimmermann HE, Dänicke S (2014) Effects of increasing concentrations of sodium sulfite on DON and DON sulfonate concentrations of different feed matrices preserved with propionic acid at various moisture contents. In progress

(2) Schwartz-Zimmermann HE, Wiesenberger G, Unbekannt C, Hessenberger S, Schatzmayr D, Berthiller F (2014) Reaction of (conjugated) deoxynivalenol with sulphur reagents - novel metabolites, toxicity and application. World Mycotoxin Journal; 7(2): 187-197

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106. Aspects of nutritional ecology including hypoglycin A concentration in various organs of the genus maple (Acer) – relevance for the sickness atypical myopathy in horses

Ernährungsökologische Aspekte einschließlich Hypoglycin A-Konzentration in verschiedenen Organen der Gattung Ahorn (Acer) – Relevanz für die Erkrankung Atypische Myopathie bei Pferden

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1060 horses on pastures died in Europe between 2006 and 2012 of atypical myopathy, 173 horses in autumn 2014 (1). Since the nonproteogenic aminoacid hypoglycin A in the Acer seeds is known to be the etiologic agent (2), from point of view of nutritional ecology the question arises why 'there are many examples of horses coexisting with these maple species […]' (3).

Methods: 19 cases of atypical myopathy on 13 pastures in Germany from October 2013 to May 2014 were investigated. The sickness was either proven by the metabolite methylenecyclopropylacetic acid of hypoglycin A in the serum (9 cases) or by other unequivocal symptoms (10 cases). On the 13 locations we recorded height, composition and ingested species of the vegetation. In three companion horses free of symptoms the grazing behaviour was filmed. Monthly mean temperatures, wind velocity, and precipitation were drawn from the Deutscher Wetterdienst and SYNOP from 1999 to 2014. 50 plots of 1 m² in an area with two old maple trees were established in which seeds and seedlings were counted weekly from October 2013 on. As to the content of hypoglycin A, various organs of A. pseudoplatanus and A. campestre from seven pastures as well as from other sites were submitted to a trial with five variants: 1. Control (Storage under constant temperatures around 18°C since the harvest), 2. Warm short-term (4 days 8°C, 1 day 25°C), 3. Cool long-term (5 days 8°C), 4. Frost short-term (4 days 8°C, 1 day -15°C), and 5. Frost long-term (5 days -15°C).

Results: On the heavily grazed areas the vegetation height was below 10 cm. No species of other taxa containing hypoglycin A (Sapindaceae, Hippocastanaceae) grew at the locations. 10 of 19 cases of atypical myopathy happened during October, the period of the main seed fall. In two cases A. campestre, in the rest A. pseudoplatanus (Sycamore) was involved. Some 60 % seeds germinated from March to April when the two last cases occurred. Horses avoided the adult leaves of all Acer species (fallen ones, seedlings), however, ingested the first leaves (cotyledons). The monthly mean temperature during autumn in 2013 of 13.7°C was not different to that of the decade ago. Neither was there a particular coincidence of strong wind/low precipitation. Ripe seeds of A. pseudoplatanus from the pastures, collected in November 2013, and stored seven months under room temperatures, showed a median of 786 nmol hypoglycin A/g (control) while unripe ones from July 2014 contained 1946 to 2583 nmol/g. Seeds of A. campestre, sampled in December 2013 on a shadowed pasture, possessed a significant lower hypoglycin A content (4.61 nmol/g; control) than those of a sun exposed paddock collected in April 2014 (925.5 nmol/g). Apart from the tendency that the hypoglycin A content decreased in unripe seeds of both Acer species after long frost, no significant difference was found between the variants of the temperature trial. The absolute maximum of hypoglycin A was measured in the first adult leaves of A. pseudoplatanus seedlings (16890 nmol/g), followed by its first leaves (cotyledons; 14492 nmol/g). In contrast, old adult leaves of A. pseudoplatanus contained 765 nmol/g, and of A. platanoides 794 nmol/g.

Conclusion: The coincidence of high load both of seeds (early autumn) and seedlings (spring) on the one hand and the death of most of the 19 horses during these times on the other hand shows that the increased availability of certain plant organs leads to their increased accessability and ingestion, too. This is particular true for short vegetation where the seedlings and winged seeds protrude. However, large scale meteorology data presented here, did not provide evidence of any strong wind or low rainfall that could have contributed to an easy accessability. In spring, yet independently from any temperature impact before, there might be an increased risk of poisoning with A. campestre seeds since they could possess a significant high hypoglycin A content due to unknown reasons. However, grazing behavior revealed that coexistence between sycamore and horse do exist in case of adult leaves, although the first adult leaves turned out to be most toxic of all organs investigated. Grazers probably sense the high tannin content when the seedling develops its first adult leaves. The absence of first adult leaves until April could explain why the incidence of atypical myopathy rises again in early spring. Thus, not only the toxin matters, but the stage of development in the maple plant. Since seeds and cotyledons seemingly miss any antipastoral traits, it might happen by chance, more or less corresponding to the number of seeds and seedlings on the pasture, whether a horse get poisoned or not.

(1) Atypical Myopathy Alert Group, http://labos.ulg.ac.be/myopathie-atypique/database, (2) VALBERG SJ et al. (2012). Equine Vet. J., (3) GILMAN JH et al. (2014). Equine Vet. J.

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107. Concentration profiles of zearalenone (ZEN), deoxynivalenol (DON) and their metabolites in bovine blood plasma and follicular fluid

Transfer von Zearalenon, Deoxynivalenol und ihre Metabolite von bovinem Blutplasma in Follikelflüssigkeit

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Zearalenone (ZEN) and deoxynivalenol (DON) are two of the most important mycotoxins of the genus Fusarium (FUS). Due to its strong affinity to oestrogen receptors, ZEN acts as an endocrine disruptor and interferes with fertility. Even DON affects oocyte maturation and follicles. Therefore, a dose-response study was performed to investigate the relationship between the concentrations of ZEN, DON and their metabolites in blood plasma and follicular fluid (FF) in order to evaluate the usefulness of blood toxin residue levels for evaluation of the developing oocyte.

Methods: For duration of 13 weeks starting one week after calving, 30 German Holstein cows were fed a diet containing 50 % grass silage and 50 % concentrate on dry matter (DM) basis. The cows were divided into three groups fed rations with the following dietary toxin concentrations: CON (Control) 0.02 mg ZEN and 0.07 mg DON, per kg DM; FUS-50 0.33 mg ZEN and 2.62 mg DON, per kg DM; FUS-100 0.66 mg ZEN and 5.24 mg DON, per kg DM. FF was obtained from 26 cows via ovum pick-up (OPU) one week after the second ovulation post parturition. Blood samples were collected by puncture of one Vena jugularis externa shortly before the follicle puncture.

For analysis, FF and plasma were incubated with β-glucuronidase overnight at 37°C. Afterwards, the samples were purified by solid phase extraction on Oasis HLB and ZEN, DON and their metabolites were determined by LC-MS/MS. Additionally, some samples of the FUS toxin contaminated groups were analysed without incubation.

All toxin concentrations lower than the limit of detection (LOD) were set to zero and concentrations between LOD and limit of quantitation (LOQ) retained their calculated values. Because the toxin concentrations were not normally distributed the non-parametric Kruskal-Wallis test of Statistica for the WindowsTM operating system (Stat Soft Version 10.0, 2011) was used with the group as fixed factor to evaluate the data. Furthermore, the relationships between plasma and FF toxin concentrations were created using the linear regression analysis of Statistica.

Results: The median (Med.) as well as minimum and maximum of the detected toxins are presented in the Table. The concentrations of the metabolites of ZEN were lower than the LOQs. Cows fed the highest contaminated diet had significantly higher toxin concentration than cows fed the control diet. ZEN as well as DON were not detected in FF in control group and de-DON was only detected in one sample.

The mean proportion of de-DON of the sum of DON and de-DON ranged between 93 and 98 % and the degree of glucuronidation of ZEN and DON amounted 100 %, while more than 96 % of de-DON was conjugated in both specimens.

	Follicular fluid			Blood plasma		
Group	**ZEN**	**DON**	**de-DON**	**ZEN**	**DON**	**de-DON**
Median						
CON	0.00^{b}	0.00^{b}	0.00^{b}	0.08^{b}	0.57^{b}	1.27^{b}
FUS-50	0.03^{ab}	0.85^{a}	28.9^{b}	0.11^{ab}	1.24^{a}	25.9^{ab}
FUS-100	0.05^{a}	1.36^{a}	61.7^{a}	0.16^{a}	1.31^{a}	44.6^{a}
Minimum						
CON	0.00	0.00	0.00	0.07	0.32	1.00
FUS-50	0.00	0.54	17.4	0.09	0.94	13.8
FUS-100	0.04	0.00	34.4	0.10	0.34	26.4
Maximum						
CON	0.00	0.00	0.15	0.09	0.99	3.95
FUS-50	0.05	2.22	37.1	0.13	1.90	40.3
FUS-100	0.12	2.76	88.8	0.23	2.35	85.5

[ab]Values with different superscripts are significantly different within row

Conclusion: ZEN, DON and their metabolites in blood plasma and FF were detected simultaneously within a controlled feeding study. Linear relationships between toxin concentrations in plasma (x) and FF (y) were established: ZEN: y = -0.03 + 0.50*** · x, r^2 = 0.58; ***p2 = 0.84; ***p2 = 0.93; ***p

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108. Impact of tannin extracts from sainfoin and birdsfoot trefoil on *in vitro* ruminal biohydrogenation of polyunsaturated fatty acids

Einfluss von Tanninextrakten aus Esparsette und Hornklee auf die ruminale Biohydrogenierung von ungesättigten Fettsäuren untersucht in vitro

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Question: During biohydrogenation in the rumen, polyunsaturated fatty acids (PUFA) are converted into saturated fatty acids mainly by *butyrivibrio spp*. Condensed tannins (CT) might be able to decrease ruminal biohydrogenation (BH) of PUFA (1). However, it is unclear which impact the source of the CT and their concentration have on the strength of the effect. The aim of the present *in vitro* study was 1) to compare the effects of sainfoin (SF) and birdsfoot trefoil (BT) CT extracts at the same inclusion level, and 2) to determine the CT effect of SF at two inclusion levels on ruminal biohydrogenation of PUFA.

Methods: Incubations were carried out using a batch culture technique (2). A diet, containing 60% hay, 33% maize silage and 7% linseed, was ground to pass a 1-mm screen. 0.5 g of the diet and either 0 mg (CON), 2.5 mg BT extract (BT5), 2.5 mg SF extract (SF5) or 14 mg SF extract (SF28) were weighted into glass flasks. Subsequently, ruminal fluid, taken from 2 cannulated cows fed a diet composed of grass silage, maize silage and concentrate, and buffer were mixed (1:4 v/v) and 50 ml was added to each flask. Incubations were stopped after 3, 6, 12 and 24 h and residues were analysed for fatty acid composition as previously described (2). Each treatment and time point combination was incubated in triplicate in 2 runs (n=6). Data were evaluated by analysis of variance at each time point using treatment as fixed effect in the model.

Results: After 3 h of incubation, no differences (P>0.05) occurred in the fatty acid profile among treatments. Independent of the treatment, concentrations of 18:2-n6 and 18:3-n3 decreased with increasing incubation time. However, extent of BH varied among treatments because at each time point the greatest (Pt11) and end product (18:0) of BH of 18:2-n6 and 18:3-n3 increased with increasing incubation time. While the concentration of 18:0 with SF28 and BT5 was constantly lower (Pt11 was lowest with SF28 compared to CON after 6 and 12 h but became similar (P>0.05) to all treatments after 24 h of incubation. The concentration of the c9t11 CLA isomer increased linearly in the SF28 treatment with increasing incubation time. However, concentration was lower (P=0.06) compared to all other treatments after 3 h but became greater (P=0.06) compared to CON after 24 h of incubation.

Conclusion: Supplementation of CT extracts resulted in greater concentration of 18:2-n6 and 18:3-n3 after 24 h of incubation. This indicates an effect of CT on ruminal BH of PUFA which depends, in turn, on the added CT amount. Differences in the effect of SF-CT and BT-CT could not be clearly detected.

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109. Effect of conjugated linoleic acid on concentrations of fat-soluble vitamins in milk of lactating ewes

Effekte konjugierter Linolsäuren auf die Konzentrationen fettlöslicher Vitamine in Schafmilch

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Conjugated linoleic acids (CLA) are well known as milk fat-reducing feed supplements for lactating ruminants. However, effects on milk concentrations of fat-soluble vitamins are unknown although the metabolism of fat and fat-soluble vitamins is interlinked (1) and effects can be expected. This study investigated the hypothesis that CLA affect retinol and tocopherol concentrations in ewe milk.

Methods: Group-housed Merino ewes nursing twin lambs and fed a grass hay:concentrate (~ 3:2) diet were partitioned into two groups according to body weight (101 ± 13.7 kg, $P = 0.92$), lactation number (2.7 ± 1.29, $P = 0.91$) and day postpartum (5 ± 2.4, $P = 0.34$). During six weeks, the diet was supplemented with either 45 g of a rumen-protected CLA supplement (Lutrell, BASF, Ludwigshafen, Germany) containing 3.4 g of both c9, t11- and t10, c12-CLA (CLA group, n = 11) or with 45 g of a hydrogenated vegetable fat (control group, n = 12) per ewe per day. The concentrate intake was fixed (1.5 kg per ewe per day) and hay intake was recorded weekly per group. Milk samples were collected at the start of the experiment and then weekly after lambs had been separated for 2 hours from their mothers. The milk fat content was determined and feed and milk were analysed for concentrations of α-, γ-, and δ-tocopherol and for retinol by high performance liquid chromatography (2). The data were analysed using R version 3.1.1. considering treatment and time as fixed and animal as random effect.

Results: Dietary intake (mg/ewe and day; mean of six weeks) of retinol was 3.1 and of tocopherol equivalents 187 for both treatment groups. The contribution of γ- and δ-tocopherol to tocopherol equivalents was minor and α-tocopherol was the main tocopherol isomer in feed and milk. Body weight loss of ewes did not differ between treatments (on average -2.5 kg in six weeks; $P = 0.12$). As expected, feeding CLA decreased milk fat percentage (-23 %; $P < 0.001$). Milk concentrations (mean ± SD; mg/kg milk) of retinol were similar in both groups (0.27 ± 0.076; $P = 0.39$) and those of total tocopherols were in tendency higher in the CLA group (0.73 ± 0.200) as compared to the control group (0.62 ± 0.192) ($P = 0.067$). When related to milk fat, feeding CLA significantly increased both milk total tocopherols and retinol during the whole experimental period (Table 1).

Table 1. Effect of CLA on concentrations of retinol and tocopherol in ewe milk (mg/g milk fat)

Week	1	2	3	4	5	6	SE	P value		
Retinol								treatment	week	interaction
CLA	4.71	7.38	6.57	7.56	7.70	7.70	0.308	<0.001	<0.001	0.022
Control	4.68	4.57	4.88	5.51	6.09	5.73	0.308			
Total tocopherols										
CLA	12.6	19.7	17.4	18.0	19.1	20.1	0.921	<0.001	<0.001	0.012
Control	11.4	10.9	11.6	12.5	14.5	15.1	0.917			

Conclusion: We show that milk concentrations of retinol and total tocopherols were maintained or in tendency increased in CLA-fed lactating ewes as compared to control ewes and were significantly increased when related to milk fat. The underlying mechanisms should be further investigated.

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110. Effect of lauric acid as dietary fat source on broiler performance, gut morphology and gut microbes

Effekt von Laurinsäure als Futterfett auf Leistung, Darmmorphologie und Darmmikroflora von Broilern

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Medium-chain fatty acids (MCFA; chain length of 6-12 carbon atoms) have anti-microbial properties and are more effectively absorbed and metabolized as compared to long-chain fatty acids (1). *In vivo* studies in broilers are promising concerning improved performance and control of pathogens but are limited to mixtures of MCFA ≤ C12 and to dosages ≤ 1% (2). We hypothesized that dietary fats rich in esterified or free lauric acid (LA) increase broiler performance and that the underlying mechanism involves changes in gut morphology and in the population of gut bacteria.

Methods: Day-old Cobb 500 broilers were partitioned into three groups of each 60 animals and each 10 animals per cage according to their body weight (43.2 ± 3.26 g; P = 0.47) and phase-fed (day 1-9, 10-17, 18-27 and 28-35) until 35 days of age. The pelletized basal diet consisted of maize, wheat, soybean meal and a fat source (4.5, 7.0, 7.6 and 8.0 % of diet in feeding phases 1, 2, 3 and 4) and supplemented with amino acids, minerals, vitamins, organic acids and, in phases 1-3, coccidiostats. Three different fat sources were used: a fat rich in oleic (38.8 %) and linoleic acid (30.8 %) (Rümanol; Rübelmann, Viernheim, Germany) (control group), a fat in which 50% of Rümanol was exchanged by a fat rich in esterified LA (17.6 % LA; group ELA) and a fat with 50% Rümanol, 25% of ELA and 25% of free LA (17.0 % LA; group FLA). Body weight and feed consumption were determined at day 9, 17, 27 and 35. At slaughter, weights of carcass, breast muscle and liver were recorded. The triglyceride content of liver and breast muscle was determined using a commercial kit (Fluitest® TG, Analyticon). Villi height and crypt depth of embedded tissue sections of duodenum and jejunum were measured microscopically and copy numbers of selected bacterial groups were determined by real-time PCR in jejunal digesta (3). The data were analysed by one-way analysis of variance using Minitab and considering treatment as fixed and animal or cage as random effect.

Results: Average daily gain (67.2 ± 3.98 g; P = 0.51) and daily feed consumption (98.1 ± 3.74 g; P = 0.21) did not differ between groups. Carcass weight (eviscerated) (1821 ± 81.7 g; P = 0.70) and liver weight (1.9 ± 0.21 % of live weight; P = 0.93) were similar as well. However, the feed: gain ratio was lower in group FLA (1.44 ± 0.022 g/g) as compared to the control group (1.48 ± 0.033 g/g) (P = 0.036). Additionally, breast muscle weight was higher in FLA (28.9 ± 0.88 % of carcass weight) than in the control group (27.7 ± 1.13 %; P = 0.009). The triglyceride content of liver (12.3 ± 4.35 mg/g; P = 0.16) and breast muscle (20.5 ± 6.80 mg/g; P = 0.47) was similar in all groups. The villi: crypt ratio of the duodenal wall did not differ (6.30 ± 2.276; P = 0.40), but in the jejunum, it was lower in group FLA (3.47 ± 1.216) as compared to the control group (4.69 ± 1.051) (P = 0.025). Copy numbers of total bacteria, Lactobacillus, Bifidobacteria, Enterobacteria, *Escherichia coli* and *Campylobacter jejuni* were similar among treatments (P > 0.05).

Conclusion: Feeding dietary fat rich in free LA improved feed conversion rate and slightly increased breast muscle percentage. The observed effects could not be conclusively explained based on the parameters measured, but the decreased jejunal villi:crypt ratio may point to changes in gut protein or cell turnover. The nutrient absorption could have been affected as well.

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111. Influence of a partial exchange of fat in the diet by palm kernel oil on feed intake, growth and health-indicators in Ross 708 broiler chicken

Einfluss eines partiellen Austausches des Fettes im Mischfutter von Ross 708 Broilern durch Palmkernöl auf Futteraufwand, Zuwachs und Gesundheitsindikatoren

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Poultry breeders created a modern broiler selected for a rapid growth rate and enhanced muscle mass gain. Those broilers do not adequately regulate voluntary feed intake which predispose them to obesity and leads to health-related problems [1]. It has been shown that lauric acid induces a restriction of voluntary feed intake [2]. The aim of this study was to investigate if the growth of these lines could be moderately reduced by specific feeding measures without limiting the food supply. Feeding measures should not have adverse effects on feed conversion, footpad health (FPD) and the plasma concentration of alpha-1-acid glycoprotein (α_1AG; as health parameter). The question was if palm kernel oil (PKO; high in lauric acids) could meet these challenges.

Methods: A total of 120 Ross 708 (R) were randomly divided in 6 groups. Each group (20 birds/2m²) of 2-week-old chickens were reared for 28 days in boxes littered with wood shavings. Three of the six groups were fed ad libitum a diet containing a conventional plant fat mixture (12.4 MJ ME/kg diet; 192 g XP/kg diet; 66.6 g XL/kg diet; 0.82 g Na/kg diet; 7.08 g K/kg diet). The other three groups were fed ad libitum the same conventional diet in which 2 % of the plant fat mixture was substituted with PKO (12.6 MJ ME/kg diet; 186 g XP/kg diet; 67.2 g XL/kg diet; 0.81 g Na/kg diet; 6.77 g K/kg diet). Feed and water intake was measured daily. The body weight measurement and external assessment of foot pads with scores of 0-7 (0 = normal skin; 7 ≥ half of foot pad necrotic; [3]) was done weekly. Four birds of each group were slaughtered and the plasma concentration of α_1AG, as marker for bacterial and viral infection just as inflammation, was individually analysed. The differences between the groups 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: The PKO fed groups had a statistically significant lower feed intake in comparison to the conventional fed groups. The differences of the final body weight and the FCR were not statistically significant. Nevertheless, the somewhat lower final body weight of the PKO fed groups (-67 g) should be taken into account. There were no statistically significant differences concerning FPD scores and plasma concentration of α_1AG. In general, the broilers of both groups showed no evidence of health problems (α_1AG ranges between the basal levels for chicken; FPD scores in average < 2).

Table 1: Comparison of the effects of a palm kernel oil replaced diet on feed intake, growth and health of Ross 708

diet	conventional plant fat mixture	2% palm kernel oil fat mixture
feed intake (g/animal, d14-21)	3817±47.2[a]	3698±48.4[b]
final body weight (g) at d 42	2828±353	2761±387
FCR d14-42	1.56±0.01	1.56±0.01
FPD scores at d 42	1.7±0.59	1.8±0.81
alpha-1-acid at d 42	230±67.6	212±58.7

[a,b] averages differ significantly ($p < 0,05$)

Conclusion: These results confirm the hypothesis of the study that it is possible to reduce the otherwise very high food intake of conventional broilers without affecting feed conversion negatively. In practice, it would be interesting to see if this already small reduction in feed intake makes it possible to reduce the health problems which are suspected to be related with excessive high feed intake.

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112. Dietary fish oil inhibits signalling pathways involved in inflammation and endoplasmic reticulum stress in the liver of sows during lactation

Fischöl hemmt Signalwege der Inflammation und des endoplasmatischen Retikulum-Stresses in der Leber von laktierenden Sauen

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Lactating sows frequently develop signs of an inflammatory condition in the liver, such as activation of nuclear factor κB (NF-κB). Hepatic inflammation and the associated acute phase response are critical, because it impairs animals´ performance and results in systemically elevated levels of inflammatory mediators, like the acute phase proteins haptoglobin (HP) and C-reactive protein (CRP) and cytokines. In addition, the inflammatory mediators are potent inducers of endoplasmic reticulum (ER) stress, a state in which unfolded or misfolded proteins accumulate in the ER lumen, thereby, leading to activation of an adaptive response known as the unfolded protein response (UPR). Activation of this pathway is considered critical, because it plays a role in the development of liver-associated diseases, such as fatty liver. The present study aimed to test the hypothesis that dietary fish oil as a source of anti-inflammatory n-3 polyunsaturated fatty acids counteracts lactation-induced activation of NF-κB and ER stress/UPR pathways in the liver of sows. In order to investigate whether the effect of fish oil is tissue-specific, we studied the effect of fish oil in this regard also in skeletal muscle.

Methods: The experiment was performed with 20 second parity gestating sows (Large White & German Landrace). After farrowing, the sows were randomly divided into two groups of 10 animals each, and litter sizes were standardized to 8 piglets per sow. Throughout lactation until the end of the experiment the sows of the two groups received two different diets for lactating sows, which varied in the type of fat (control group: 50 g of a 4:1-mixture of palm oil and soybean oil per kg diet, fish oil group: 50 g of fish oil per kg diet). At the day of parturition, the sows were given 3 kg of the diet. From the day 1 of lactation to later lactation, the amount of diet was administered in order to meet the energy requirement of the sows (assuming daily litter gains of 2 kg). The piglets were offered a creep feed from day 15 of lactation until weaning. On day 21 post partum, samples from liver and skeletal muscle (*M. longissimus dorsi*) were taken by biopsy and blood was collected from *V. jugularis*. Means of the two groups were compared by student´s t-test.

Results: The daily feed intake during the three weeks of lactation was not different between control sows and sows administered fish oil. Body weights of the sows at day 1 post partum and day 21 post partum and gains of litters during the suckling period were similar in both groups of sows. The calculated energy balance and body weight losses of the sows from day 1 post partum to day 14 or 21 post partum did also not differ between both groups of sows. In the liver, relative mRNA concentrations of 8 NF-κB target genes and 14 ER stress/UPR target genes considered were reduced by 10-79 % and 9-58 %, respectively, in the fish oil group compared to the control group; significant differences ($P < 0.05$) between the two groups of sows were observed only for the mRNA concentrations of LBP, ICAM1, BCL2, DDIT3, DNAJC3 and HSP90B1. In line with this, plasma concentrations of HP and CRP were 10-20 % lower in sows of the fish oil group than in those of the control group (HP: 2.33 ± 0.59 vs. 2.67 ± 0.81 mg/mL; CRP: 197 ± 125 vs. 324 ± 221 µg/mL), although these differences were not significant. In skeletal muscle, the relative mRNA concentrations of none of the NF-κB and none of ER stress/UPR target genes considered were different between sows of the fish oil group and the control group.

Conclusion: Dietary fish oil is able to inhibit, at least in the liver, NF-κB and ER stress/UPR pathways. It is assumed that feeding fish oil is a useful dietary strategy to improve health and performance of lactating sows.

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113. The impact of polyunsaturated fatty acids on the receptor association of TLR4 and CD14 in murine macrophages

Der Einfluss von mehrfach ungesättigten Fettsäuren auf die Rezeptorassoziation von TLR4 und CD14

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Polyunsaturated fatty acids (PUFA) have been shown to affect cellular signal transduction thereby altering a variety of physiological processes such as immune defense and inflammation. These effects are discussed to be in part due to altered membrane receptor interactions caused by incorporation of PUFA into cellular membranes. We recently demonstrated that PUFA supplementation of murine macrophages leads to an impaired compartmentalization of Toll-like receptor 4 (TLR4) and cluster of differentiation 14 (CD14) in lipid rafts thus exerting anti-inflammatory and immunomodulatory actions. Whether the direct binding of both receptors is modulated by PUFA and to what extent has not been fully elucidated yet. Therefore, the objective of this study is to investigate and quantify the association of TLR4 and CD14 following PUFA uptake.

Methods: Cells of the murine macrophage cell line RAW264.7 were cultured for 72 hours in medium containing 15 µM alpha-linolenic acid (LNA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA) or arachidonic acid (AA), respectively. Subsequently cells were stimulated for 24 hours with either LPS or viable *Pseudomonas aeruginosa* (ATCC 10145). Unsupplemented and unstimulated macrophages served as reference. Cells were harvested and lysed. Equal amounts of lysate were co-immunoprecipitated with an anti-CD14 antibody. Proteins were separated by SDS-PAGE and detected by western blotting using either anti-TLR4 or anti-CD14 antibody. Densitometric analysis from four independent experiments were performed using the image analysis software GeneTools (Syngene, Cambridge, UK). The ratio of TLR4 to CD14 was calculated to normalize for differences in loading. The degree of interaction was determined by comparison of the densitometric values of normalized TLR4. Statistics were performed using t-test.

Results: The co-immunoprecipitation experiments showed a direct interaction between the receptors TLR4 and CD14. Both receptors were slightly associated without any stimulation. The degree of interaction increased with the particular stimulus. Supplementation with PUFA attenuated receptor association in stimulated and unstimulated cells. This effect is most likely mediated through perturbation of the lipid bilayer.
These results are preliminary (as of January 2015) and subject to completion thus no final quantification has yet been made.

Conclusion: The immunomodulatory actions of PUFA are partly exerted at the membrane level due to an altered receptor association. Therefore PUFA may be beneficial for pathogen defense and pathogen-associated inflammation by inhibiting TLR4 signaling.

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114. Dietary supplementation with conjugated linoleic acid during early lactation in dairy cows: effects on the concentrations of vitamin E congeners in serum and liver

Supplementierung mit konjugierten Linolsäuren während der Frühlaktation: Effekte auf die Konzentrationen von Vitamin E-Kongeneren in Serum und Leber

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Vitamin E in its natural form comprises eight different compounds, i.e. α-, β-, γ-, and δ-forms of each tocopherol (T) and -tocotrienol (T3). The most abundant form of tocopherols, α-tocopherol (αT), is the only form used to supplement animal feed. However, T3 exhibit some unique physiological functions that are not entirely shared by tocopherols (1). In contrast to αT, little is known about plasma and tissue concentrations of T3 in humans and animals. The increase in vitamin E status of laboratory animals supplemented with conjugated linoleic acids (CLA) attracted interest in the interaction between vitamin E and CLA (2). Thus, our study aimed to characterize the concentrations of the different vitamin E congeners in serum and in liver and the hepatic gene expression of factors related to vitamin E metabolism in dairy cows during early lactation, and to test the effects of a dietary supplement with CLA.

Methods: Twenty one pluriparous German Holstein cows were randomly assigned to receive either 100 g/d CLA (n = 11; Lutrell pure, BASF, Germany; each 12% of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA) or a control fat supplement (Silafat, BASF; CTR; n = 10) from days in milk 1 to 182. Blood samples and liver biopsies were collected on d -21, 1, 21, 70, and 105 (liver only) relative to calving. Serum and liver concentrations of vitamin E congeners were quantified by HPLC. The mRNA abundance of α-tocopherol transfer protein (TPP), α-tocopherol associated protein (TAP), and cytochrome P_{450} 4F2 (CYP4F2) were quantified by real-time RT-PCR. Data were analyzed by the MIXED model with treatment, time, and interaction of treatment and time as the fixed effects and cow as the random effect.

Results: In the CLA group, mean dry matter intake (21.2 ± 0.24 kg/d; mean ± SEM) did not differ from the CTR group (22.3 ± 0.24 kg/d). There were no significant differences in any of the serum concentrations of the various forms of vitamin E between the CTR and the CLA group. The serum concentrations of αT, γT, βT3, and δT3 changed over time ($P < 0.01$) and followed a similar pattern in both groups, i.e. showing an increase from d -21 to d 21 and remaining largely unchanged between d 21 and d 70. No CLA by time interactions were observed for the serum concentrations of vitamin E forms except for γT3 ($P = 0.06$). The molar ratio of the serum vitamin E isoforms to cholesterol was not affected by the CLA supplementation. The molar ratio of all forms of vitamin E to cholesterol in the serum changed during the course of the study ($P \leq 0.02$). There were no differences in any of the liver concentrations of various congeners of vitamin E between the CTR and the CLA group. Time-related changes in the liver concentrations of the vitamin E forms were noted in both experimental groups ($P < 0.05$; $P = 0.07$ in case of γT3). The hepatic mRNA abundance of genes related to vitamin E metabolism did not differ between the two groups. In the CTR group, TTP mRNA increased during the course of the study from d -21 to 1.62-fold values on d 105 ($P < 0.01$). There was a trend observed for the interaction between treatment and time for the mRNA abundance of TTP ($P = 0.10$). In the post partum period, the abundance of mRNA encoding TAP was greater ($P < 0.001$) on d 105 than on d 70 (2.80- and 2.70-fold for the CTR and CLA group, respectively). The mRNA abundance of CYP4F2 did not change over time and there was also no treatment by time interaction.

Conclusion: All four congeners of T3 were detected in serum and liver of dairy cows during late gestation and early lactation, albeit at distinctively lower concentrations than αT and γT. Increasing mRNA expression of TPP with days in milk in the CTR group may point to an involvement of TTP in the increase of αT concentrations in the serum. Finally, our data indicate time-dependent changes in the serum and liver concentrations of the vitamin E congeners and in the hepatic expression of genes related to vitamin E metabolism that were largely unaffected by CLA supplementation.

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115. Is stomach passage a barrier against specific *Escherichia coli* (responsive for post weaning diarrhoea) experimentally applied to weaned piglets?

Stellt der Magen tatsächlich eine Barriere gegen experimentell an Absetzferkel verabreichte Escherichia coli dar? – Ergebnisse der experimentellen Infektionen und in-vitro Inokulationen

von und zur Mühlen F., Sander S., Verspohl J., *Kamphues J. - Hanover

Introduction: In today's pig production diarrhoea caused by *E. coli* is of clinical relevance with regard to losses and reduced gains. The protective effects of coarsely ground diets regarding infections with *Salmonella spp*. and *E. coli* were shown in different studies (1, 2). The part of stomach barrier function within these effects was shown with *Salmonella Derby* and *Streptococcus suis* (3). The aim of this study was to evaluate whether stomach barrier function can also have an effect on the survival rate of diarrhoea causing *E. coli* after artificial infection and in-vitro inoculation.

Methods: 18 weaned barrows were individually housed and fed a botanically and chemically identical diet (13.8 MJ ME, 237 g XP/kg 88 % DM, 42 g XF/kg 88 % DM). Diets: coarsely ground meal (CM), finely ground and pelleted (FP). After five weeks of feeding four piglets out of each group were artificially infected with an oral dose of 1.5 x 10^9 CFU *E. coli* (F4, STI, STII, LTI). The piglets were euthanized 2 hours after infection and digesta samples were taken. Remaining not infected piglets were euthanized, stomach content was quartered and set aside for in-vitro study. Therefore 1 ml bouillon of *E. coli* (1.3 x 10^9 CFU) was added to 10 g of stomach content (fundic region) and incubated in a shaking water bath (37 °C). Counts of *E. coli* in digesta samples as well as in the substrate of in-vitro study were measured by quantitative cultural techniques (serial dilution, growing on *Columbia Agar* with 5 % sheep blood, incubation at 37 °C for 24 h). Statistical analyses were done by using the SAS software (analysis mixed models, respectively, $p \leq 0.05$).

Tab1: Counts of supplied ***E. coli*** (log/g digesta*)[1]			b=bouillon, m=stomach, dd_1-dd_3=cran., med., caud. third of small intestine, cae=caecum; [1]x ± sd; *no detection=half detection limit (1.00 log)
loc.	FP	CM	
b	$9.17^{a} \pm 0.053$	$9.13^{b} \pm 0.044$	
m	$6.83^{a} \pm 0.329$	$6.43^{a} \pm 0.329$	
dd_1	$6.94^{aA} \pm 0.298$	$5.40^{aB} \pm 0.224$	
dd_2	$7.17^{a} \pm 0.333$	$6.58^{a} \pm 0.535$	
dd_3	$8.06^{a} \pm 0.093$	$8.00^{b} \pm 0.491$	
cae	$4.12^{b} \pm 2.20$	$1.79^{c} \pm 0.798$	

Results: While stomach content was firm and layered with feeding CM, the FP-group showed fluid ingesta and pH-value also differed between the two groups. There was no *E. coli* in the stomach content before inoculation. 1.5 h after oral intake *E. coli* were found in the stomach and in every part of the small intestine, in some individuals the applied *E. coli* did not reach the caecum. The highest amount was found at the end of small intestine. In-vitro the survival rate of applied *E. coli* ranged from 86 to 91 % irrespective of the time of incubation.

time	FP[1] (pH=3.89-4.72)	CM[1] (pH=2.80-4.55)	Tab2: Relative proportion of ***E. coli*** after in-vitro inoculation
bouillon	1.00 ± 0.00	1.00 ± 0.00	
a. inoc.*	-*	-*	
3 min	0.86 ± 0.02	0.88 ± 0.01	*no detection of E. coli before inoculation; [1]x ± sd
60 min	0.87 ± 0.01	0.88 ± 0.02	
120 min	0.91 ± 0.09	0.86 ± 0.01	
240 min	0.90 ± 0.10	0.87 ± 0.03	

Conclusion/discussion: The orally applied bacteria reached the terminal ileum and even caecum with no statistically significant effects of dietary treatment. That could be managed by a transfer within the liquid phase of gastric content. In fundic region as the most acid part of stomach some bacteria cannot survive (3) but this strain of *E. coli* survived (90 % recovery rate). The stomach barrier seems to have less impact on *E. coli* because diarrhoea causing *E. coli* survived even under the condition of a well-established stomach barrier and layered content (group CM). Thus other defence mechanisms might be needed to protect the piglets after rearing but the question arises how bacteria/toxins can be "inactivated".

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116. Fattening performance and carcass conformation of dual purpose poultry genotypes in comparison to broiler and layer genotypes

Mastleistung und Schlachtkörperkonformation von Zweinutzungsgenotypen im Vergleich zu Broiler- und Legegenotypen

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Currently there is an ethical discussion about the practice of culling 1-day old male layer chickens. Two German federal states want to start prohibiting this practice. Therefore, there is a need for dual purpose genotypes satisfactory for both egg and meat production. However, the expected lower feed conversion efficiency (FCR) of non-broiler genotypes is of concern. In addition, the consumers' purchase decisions will be influenced by carcass conformation both when marketed as entire carcasses (especially a prominent keel bone is unappealing) and when breast meat is sold alone (small, flat cuts). In the present study, three dual purpose genotypes were compared with both broiler and layer genotypes in fattening performance and carcass conformation in order to quantify whether dual purpose genotypes are really superior to male layer genotypes and may at least compete with slower growing broiler genotypes.

Methods: A total of six genotypes were compared with two positive controls (a fast growing broiler line Ross PM3, C++; a slower growing broiler line Sasso 51, C+), a negative control (layer genotype, bred for the organic market, Lohmann Brown Plus, C-) and three dual purpose genotypes (Lohmann Dual, LD; the Belgian Mechelner breed, ME; the Swiss Schweizerhuhn, CH). Nine birds per genotype were investigated, either kept in pairs in cages of 0.6 m^2 (n=3 for feed-intake related data) or in boxes of 1.7 m^2. All birds received the same intensive broiler fattening diet (calculated contents of metabolisable energy and crude protein: 13 MJ and 198 g/kg as fed). C++ were fattened for the conventional period of 5 weeks, the other genotypes for 9 weeks as prescribed for organic farming in Switzerland. Body weight (BW) and feed intake were determined weekly. At slaughter, carcass and breast meat were weighed and breast meat yield (BMY) was calculated as the ratio of breast meat weight on carcass weight. Data were subjected to ANOVA using SAS 9.3 with genotype as fixed effect. For multiple comparisons of the least square means the Tukey-Kramer option was used.

Results: C++, C+ and LD reached the highest final BW, ME was intermediate and C- and CH were lowest (Table 1). Differences in average daily gains (ADG) were the same except for C++ which was highest. FCR was best with C++, followed by LD, intermediate with C+, ME and C- and most unfavourable with CH. Dressing percentage was highest for C++, C+ was higher than LD, ME and CH, C- was lowest. C++ had the highest BMY followed by LD and C+, and then CH, ME and C-.

Table 1: Effect of genotype on growth performance and carcass traits

	C++	C+	C-	LD	ME	CH	SEM	P-value
Final BW (g)	2415^{a}	2429^{a}	1232^{c}	2164^{a}	1762^{b}	1323^{c}	80.1	<0.001
ADG (g/d)	68^{a}	38^{b}	19^{d}	34^{b}	27^{c}	20^{d}	1.4	<0.001
FCR (g feed/g ADG)	1.5^{d}	2.5^{b}	2.5^{b}	2.3^{c}	2.6^{b}	2.8^{a}	0.04	<0.001
Dressing percentage	73^{a}	69^{b}	63^{d}	67^{c}	66^{c}	65^{c}	0.5	<0.001
BMY(% of carcass)	30^{a}	20^{b}	17^{c}	19^{b}	17^{c}	17^{c}	0.4	<0.001

$^{a-d}$Means within a row carrying no common superscript are significantly different ($P<0.05$).

Conclusion: None of the dual purpose genotypes performed as well as the intensive broiler line, but LD could compete with a more extensively growing broiler line (slower growth, better FCR, similar BMY), but they performed in a more heterogeneous way. The genotype CH was only at the level of the layer genotype. Further analysis will focus on meat quality.

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117. Natural Campylobacter infections in broiler fattening - is there an influence of dietary concepts on prevalence and extent of infection

Natürliche Campylobacter-Infektionen in der Broilermast – gibt es einen Einfluss von diätetischen Konzepten auf Prävalenz und Ausmaß der Infektion

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Campylobacter jejuni - infections are the leading cause of bacteria-induced intestinal diseases of humans in Germany. Poultry meat is regarded as the main risk food. There are initial studies on influences of certain feeding measures (fatty acids, etc.) on the prevalence of Campylobacter (*C.*) infections in poultry (1), but often only in vitro. In this study influences of different dietary concepts (protein source/fat/coccidiostats) are examined in relation to the extent of a *C.* infection.

Methods: A total of 340 broiler line Ross 708 from one hatchery were reared together during the first week (feed, box, etc.) and then divided into 17 groups of 20 animals (2 qm / box, littered with wood shavings, one room). From day 14, overall 7 different basic rations were fed up to dissection on days 42/44. These were on the one an own made mixed feed with four protein combinations (4x2 groups; soybean meal=SBM; WISAN®Raps=WIS; haemoglobin meal= HBM; Chlorella Vulgaris-powder=ALG) and soyabean oil, on the other hand, 3 fat supplements in a SBM/-based industrially produced mixed feed (fA-C; 3x3) containing 10 % whole wheat (coarsely ground, added after pelleting) and Monensin-Na as coccidiostat. The presence of *C.* in pooled excreta samples was investigated during fattening. At dissection (d 42/44) the number of *C.* species in the caecal digesta was determined. These investigations were carried out in accordance with § 64 LFGB 0:00 107.

Table 1: Experimental set-up in terms of ingredients (types of protein etc.) and feed composition

group	SBM	WIS	HBM	ALG	SBM-fA[1]	SBM-fB[2]	SBM-fC[3]
SBM (%)	32.5	22.9	22.5	28	25.3	25.3	25.3
further protein source	-	14.5	4.5	4.0			
ME (MJ/kg diet)	12.1	12.0	12.7	12.6	12.4	12.6	12.4
XP/XL (g/kg diet)	208/71.8	205/75.9	213/75.4	211/80.2	192/66.6	186/67.2	189/63.0
Ca/P/ (g/kg diet)	12.5/4.5	13.3/6.4	14.2/6.2	12.9/6.1	5.2/4.5	5.5/4.3	5.5/4.5
Na/K (g/kg diet)	1.8/8.8	1.9/8.0	1.8/6.9	1.8/8.3	0.8/7.1	0.8/6.8	0.8/6.9

[1] mixture of fat; [2] mixture of fat including 2 % palm kernel oil (2%); [3] mixture of fat including 2 % of medium chain fatty acids

Results: *C.* was detected much earlier and more frequently in the groups without coccidiostats and whole wheat in the compound feed. In samples from the caecum content in these groups, the prevalence was higher as well as the *C.* counts were also increased.

Table 2: Detection rate of *C.* in pooled faeces samples (d17-d39) and caecum content (n-pos./n-total) and *C.* counts (cfu/g) in the caecum content

time	SBM	WIS	HBM	ALG	SBM-fA	SBM-fB	SBM-fC
d 17	-/2	-/2	-/2	-/2	-/3	-/3	-/3
d 24	2/2	2/2	2/2	2/2	-/3	-/3	-/3
d 33	1/2	0/2	2/2	2/2	-/3	-/3	-/3
d 39	2/2	0/2	2/2	2/2	-/3	-/3	1/3
dissection	10/10	9/10	10/10	10/10	7/15	6/15	7/15
caecum	7.49±1.87	6.69±3.01	7.04±1.72	8.21±1.20	2.47±3.06	2.05±2.83	3.15±3.97

Conclusion: Inspite of housing in one room, chicken from hatchery etc. in groups of chicken fed the conventional diet (with coccidiostats, with 10 % roughly shredded wheat, lower XP and mineral content) *C.* bacteria were detected rare and in less counts so that a feeding influence can be assumed.

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118. Are fattening turkeys of modern lines affected by a Hashimoto like thyroiditis?

Leiden B.U.T.6 Mastputen an einer Hashimoto-ähnlichen Thyreoiditis?

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Hashimoto´s Thyroiditis is one of the most common reasons for hypothyroidism in human patients. However it has not yet been reported to occur in fast growing turkeys. The present finding was an accidental one and observed in the context of a study, which was conducted to assess the effects of high levels of rapeseed meal (RSM) on the performance of male turkeys. Thyroids were examined to detect any goitrogenous effect of the fed RSM.

Methods: The experiment consisted of 6 fattening phases lasting 133 days in total. Four hundred and twenty, one day old, male B.U.T.6 turkey chicks were reared in the poultry facility of the University of Applied Sciences Weihenstephan-Triesdorf. RSM replaced SBM with rising amounts of 5% in phase 1, 2, 3 and 5. A RSM free diet, with SBM as a protein source was fed to the control group. The glucosinolate content of the used 00-RSM amounted to 7.69 µmol/g. The total iodine contents in diets amounted to 2 mg/kg throughout all phases. Given that the feed mixtures did not comprise any coccidiostats, a second control group (the commercial standard group=CS), receiving a common industry diet with monensin-sodium as a coccidiostat from phase 1 to 4 and low doses of RSM from phase 3-6 was kept as well. Thyroid-samples were dissected after slaughter from 12 animals of each group and 8 of group CS, respectively. Post mortem tissue samples were examined histologically. As no goitrogenous effects could be detected, but lymphocytic infiltrates occurred independend of the dietary treatment, the severity of lymphocytic thyroiditis was scored (0=no microscopic lesion to 4=severe, coalescing to diffuse lymphocytic thyroiditis). To characterize the infiltrating lymphocytes the thyroid glands of 12 animals (3 of scores 1 to 4) were investigated immunohistochemically. Blood samples could not be taken, because this finding was accidental. The collected individual animal data were evaluated statistically using the SPSS V.20 (2011) programme according to the „General Linear Model“, which was used for the factor RSM in single feed. Deviations were examined using the Tukey-Test and Chi-square Test.

Results: Growth, feed intake and conversion ranged in all groups above the common levels in the field. Losses were comparatively low (rearing period: 3.6%, fattening period 1.85%). In all groups there was a high incidence of lymphocytic thyroiditis. Altogether 14% of the examined thyroids showed a moderate to severe lymphocytic thyroiditis. There was no systematic effect of feeding group. Immunohistochemistery identified an inflammatory infiltrate characterized by predominantly T-cells admixed with scattered B-cells in all animals tested. Occasionally, accumulations of B-cells were indicating germinal center development. The severe form of thyroiditis seen here seems to resemble Hashimoto´s disease in humans (1). Wick, et al. (1974) (2), demonstrated the possibility of such a disease in poultry. Given the high productivity and growth intensity of the turkeys a severe hypothyreosis is unlikely. A mild hypothyreosis, however, might increase feed conversion because the maintenance requirements may be reduced due to lower metabolic rate and activity. In human nutrition it is known that an overload of iodine in food can cause an onset of autoimmune thyroiditis in predisposed individuals. Currently there is a wide range between iodine recommendations in diets of fattening turkeys (0.4 mg/kg to 2 mg/kg) (3). Consequently an enhancing effect of high or varying iodine contents in diets of fattening turkeys cannot be excluded.

Conclusion: We hypothesize that there might be a disposition in fast growing turkey lines towards a mild Hashimoto-like hypothyreosis. However in birds the dissemination of lymphocytic tissue is not unusual, so that only score 3 and 4 should be taken in account as indicators for an autoimmune thyroiditis. The effects of high iodine contents in diets of fattening turkeys as a possible trigger for the onset of an autoimmune thyroiditis need further investigation. Besides the potential effects of such a disease on current health problems (pododermatitis, cardiovascular problems, breast buttons and blisters) in the intensive production system of fattening turkeys need to be analysed as well.

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119. Ontogeny of the circulating adiponectin concentrations in neonatal calves: the importance of colostrum intake

Ontogenese der Adiponectinkonzentrationen im Blut neugeborener Kälber: die Bedeutung der Kolostralmilchaufnahme

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Adiponectin, one of the most abundant adipokines in circulation, has a role in nutrient partitioning through its insulin-sensitizing actions. It is mainly expressed in adipose tissue and the circulating concentrations are inversely associated with adiposity and inflammation (1). Adiponectin is also present in milk (2) and thus the question arises whether adiponectin from milk and colostrum might be important for the development of the offspring. Moreover, research on the role of adiponectin was largely focused on lactating cows whereas young prepubertal calves were not studied in this respect. We thus aimed to characterize the adiponectin concentrations in neonatal and young calves that were fed with either colostrum or formula.

Methods: Two trials were performed: in Trial 1, German Holstein calves (n = 14) were studied for the first 4 d of life. They were bottle fed either with pooled colostrum (**C**) or milk replacer with comparable nutrient composition as colostrum (**MR**). Colostrum and MR amounts fed were targeted to be 8% of BW on d 1 and 10% of BW on d 2 to 4. Blood samples were taken right after birth and before each morning feeding on d 2, 3 and 4. In Trial 2, 20 German Holstein calves were all fed with colostrum from their dam (2 times/d) for 3 d, and then received MR (130 g/L) for 56 d and were weaned thereafter to a total mixed ration. Blood samples were collected on d 0 (before colostrum feeding), and d 1, 3, 11, 22, 34, 43, 52, 70, 90, 108 *post natum*. Adiponectin was quantified by an ELISA specific for bovine adiponectin with minor modifications (3); statistical evaluation was done using the mixed model with time (and treatment, for Trial 1 only) as fixed factors and calf as repeated subject.

Results: In the Trial 1, before colostrum consumption, serum adiponectin concentrations were low in the C group (2.83 ± 0.30 µg/mL), comparable with the MR group (2.72 ± 0.53 µg/mL), but were 3.9-fold increased at 24 h after colostrum intake (10.8 ± 0.80 µg/mL; $P < 0.05$) without any further change until d 4. The serum adiponectin concentrations of the MR group remained unchanged at 24 h after MR intake, but slightly increased until d 4 (5.12 ± 0.45 µg/mL; $P < 0.05$). The calves in the C group had consistently greater ($P < 0.05$) blood adiponectin concentrations than the MR calves, except after birth and before first feeding of colostrum, which was the same in both groups. When following the time course of adiponectin blood concentrations in the colostrum fed calves from Trial 2 until d 108 of life, 3.5- and 4.7-fold lower values were recorded before colostrum intake (d 0) than thereafter in the d 1 and d 3 samples, respectively (2.60 ± 0.25 vs 9.13 ± 0.44 and 12.1 ± 0.65 µg/mL; $P < 0.05$). Until d 52, the concentrations remained unchanged, but increased again thereafter until the end of the study on d 108 of life ($P < 0.05$).

Conclusion: Adiponectin blood concentrations in newborn calves clearly depend on the intake of colostrum. The increase observed with colostrum intake points to a direct transfer from colostral adiponectin into the circulation of the newborn. The increase in the blood adiponectin concentrations in calves after weaning is most likely due to increasing endogenous production of adiponectin that might have been triggered, at least in part, by the complete change to a diet of solid feeds.

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120. Locomotive and feeding behaviour as well as growth performance of beef calves on seasonal mountain pastures: influence of the presence of adult females

Bewegungs- und Fressverhalten sowie Wachstumsleistung von Kälbern auf alpinen Weiden: Einfluss der Anwesenheit von adulten weiblichen Tieren

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Previous research showed that suckler calves on steep alpine pastures walked slightly more than suckler calves on flat pastures and had moderately lower weight gains (1). The presence of the dams of the calves could have limited differences because the availability of the dam's milk might reduce the need of the calves to browse through the steep pasture. In addition the calves could learn from adults how to graze economically efficient, even if the accompanying adults are not their dams. The aim of the present experiment was to identify the role of the dam still providing milk and of adult females in general on the calves' feeding behaviour, physical activity and growth performance on steep alpine pastures.

Methods: Twenty-four Charolais-sired suckling calves (born between January 15th and March 15th) originating from one Angus×Holstein cow herd were distributed over three groups of eight animals each balanced for live weight (187 ± 23 kg) and age (3.3 ± 0.5 months). Thereof 2/3 of all animals were weaned on May 7th. The three treatments consisted of: weaned calves without any contact with adult animals (W), weaned calves in a herd of eight mentoring dry cows known to the calves (Wm), and calves still suckling their dams (S). The dams from W calves, dried off on May 7th, were kept apart from calves until June 12th, when they were combined to one herd with the Wm calves. This period served to avoid suckling by the Wm calves on the adult females. In addition, nose rings were fitted on the Wm calves during the first weeks. On June 19th, all experimental animals were moved to seasonal alpine pastures (20-35% inclination) of the ETH located around 2000 m a.s.l. in the Swiss Alps. The pastures had a known similar feeding value and were managed in a rotational grazing system. Locomotive and nutritional behaviour of the calves were monitored using IceTag pedometers and MSR rumination sensors. Live weight was measured weekly to fortnightly. The data were subjected to analysis of variance with SAS 9.3.

Results: W and Wm calves spent more time eating and ruminating than S calves ($P<0.05$ and $P<0.07$; respectively). Pedometer data indicate a higher activity for weaned calves. Especially the Wm calves walked more than the S calves (3679 vs. 3052 steps/day; $P<0.01$) and spent also less time lying (11.3 vs. 13.1 h/day; $P<0.01$). For herd size reasons, W calves had paddocks five to ten times smaller than Wm and S calves. Nevertheless, this did not result in a fully proportionate decline in locomotive activity (2529 steps/day) compared to the Wm calves. W calves lied down for a similar time (11.1 h/day) as the Wm calves. There was a trend ($P=0.07$) towards a lower live weight of W and Wm calves compared to S calves before transport to the alpine site on June 12th (208 and 208 vs. 239 kg) and differences increased over time until slaughter on September 1st (251 and 238 vs. 344 kg; $P<0.01$). Live weight never differed between W and Wm calves.

Conclusion: Weaning immediately affected the live weight development of the calves to some extent. This effect increased under the difficult conditions of alpine pastures. The higher locomotive activity of the Wm calves did not result in a lower growth performance compared with the W calves. The W calves were observed to be restless compared to the two other groups which might have contributed to the present results. The greater calmness of the Wm calves might have been either the result of learning from the adults or simply the reassuring effect of the presence of mentoring adults.

(1) GANGNAT et al. (2014): Proc. Aust. Anim. Prod. 30: 171.

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121. Effect of rearing intensity during early postnatal life on long-term pancreatic ß-cell development in Holstein-Friesian bull calves

Einfluss der Aufzuchtintensität während der frühen postnatalen Phase auf die langfristige Entwicklung der ß-Zellen im Pankreas von männlichen Holstein-Friesian Kälbern

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A permanent effect of early postnatal development on growth and function of tissues and organs has been reported for various mammalian species. The objective of this study was to assess the impact of two different rearing strategies during the first 3 weeks of life on insulin producing pancreatic ß-cells in calves.

Methods: During the first 3 weeks of life Holstein-Friesian bull calves were fed either intensively (INT; ad libitum whole milk feeding; individual hutches; N=21) or according to an established restrictive rearing protocol (CON; 4 L whole milk/d during week 1 in hutches, 720 g/d milk replacer from days 8-21 in group pens; N=21). From day 22 of life, all calves were housed and fed similarly until slaughter at an age of 8 months. Birth weight and weekly body weights up to week 10 were recorded. Plasma glucose, insulin, insulin-like growth factor-I (IGF-I) and growth hormone (GH) levels were assessed during wk 1, 2, 3 and 10 of life. At slaughter, tissue from the medium body of the pancreas was removed and fixed in formaldehyde. By immunohistochemistry, the number of pancreatic islets of Langerhans per microscopic field of view was assessed manually (AxioPhot, AxioCam and AxioVision 4.5, all Zeiss, Germany) and the total area of insulin stained cells per photograph was obtained using a photomicroscope and a picture processing software program (Eclipse E600, DS-Fi1, NIS-Elements Basic Research 3.2., all Nikon, Düsseldorf, Germany). Statistical analyses of feed intake, body weights and blood results (MIXED procedure in SAS) were performed including group, week of life and the interaction group x week of life as fixed effects and animal as random effect. The analyses of number of islets of Langerhans and insulin stained area per photograph (MIXED procedure in SAS) included group as fixed effect, birth weight as covariate and animal as random effect. Bonferroni post hoc tests were performed and data are presented as LSMeans ± SEM.

Results: The birth weight and the intake of colostrum was comparable between groups ($p>0.05$ each). Total energy and crude protein intakes of INT calves were nearly double the intakes in CON calves in the first 3 weeks of life ($p<0.01$). Daily concentrate intake during week 4-10 of life did not differ between groups ($p=0.24$). The average daily gain was higher in INT-calves compared to CON calves during the first 3 weeks of life (1.28 *vs.* 0.38 kg/d; $p<0.001$), but weight at slaughter did not differ (319 *vs.* 309 kg; $p=0.18$). INT calves displayed increased blood glucose, insulin and IGF-1 concentrations in week 3 of life compared with CON calves (all $p<0.05$), whereas GH was lower in INT calves during the 2nd week of life. In week 10 of life glucose, insulin, GH and IGF-1 concentrations in plasma did not differ. In INT calves, more islets of Langerhans per microscopic field of view compared to CON calves were found (9.1 ± 0.3 *vs.* 7.8 ± 0.3; n=6-18 per calf; $p<0.01$). The total insulin stained area per photograph was larger in INT calves compared to CON calves (0.107 ± 0.005 mm^2 *vs.* 0.084 ± 0.005 mm^2; n=5 per calf; $p<0.01$).

Conclusion: Intensive rearing during the first three weeks of life results in an enhanced nutrient uptake and body development in comparison with a conventional rearing protocol. The observed alterations in pancreatic insulin producing tissue imply that the rearing intensity during the neonatal period has long-term consequences which supports the theory of a possible metabolic programming in intensively reared calves. Consequences for future performance particularly in dairy cows warrant further investigations.

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122. **Metaproteomics: analysing the active fraction of the broiler gut and rumen associated microbiota**

Metaproteomics: Analyse der aktiven Fraktion der Darm- und Pansen-assoziierten Mikrobiota

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The microbiota of the animal's gastrointestinal tract plays an essential role for the growth and health of the animal. It is well accepted that the structure and function of the microbiota is influenced by outer parameters like composition of the feed, addition of pro-, pre- and antibiotics, environmental factors as well as the by animal itself. Gathering knowledge about the activity and function of the gut microbiota under different feeding conditions can be important to develop highly adapted animal feed in order to increase the efficiency in livestock production and decrease the emission of undesired substances by ruminants for example. The identification and assessment of the protein inventory of the microbiota is named metaproteomics and displays a valuable interface between gene-based analysis and metabolomics studies. Metaproteomic studies of gut samples or feces are challenged by a high fraction of eukaryotic proteins, which originate from feed particles and host cells and are co-extracted during the sample preparation procedure. Samples from the crop and ceca of broilers and rumen samples were used to improve sample preparation strategies and to give a first impression of the active microbial fraction in these gut sections.

Methods: Crop and ceca digesta samples of broilers originated from a dietary treatment based on a two maize-soybean meal basal diets (BD); one containing mineral phosphorus (P) exclusively from plant sources (BD-), the other supplemented with P from monocalcium phosphate (MCP) (BD+). Treatments consisted of the BD- and BD+ supplemented with 0, 500 or 12,500 phytase units/kg [1]. A modified protocol from [2] was used to disaggregate the cells in the samples by a chemical/physical treatment followed by multiple steps of centrifugation and resuspension, yielding a fraction enriched with prokaryotic cells. Proteins were extracted using a detergent and sonication based lysis procedure. Proteins were 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. Solid (RS) and liquid (RF) rumen samples were obtained from a fistulated Holstein-Frisian cow fed twice a day with 4.5 kg hay diet containing 12% concentrate. Two protocols were tested to separate prokaryotic cells from feed particles in the RS samples: One using detachment buffer including 0.1% methylcellulose followed by a Histodenz density-gradient centrifugation step (RS-H). The other one used the detachment buffer and a cheesecloth gauze (RS-C). Protein extraction and following procedures were performed as described above.

Results: The protein identifications of the broiler samples revealed that the active fraction of the crop-microbiota had a low bacterial diversity with *Lactobacillaceae* as the dominant family. Bacterial protein identification was still limited due to high a number of proteins derived from broiler feed. The ratio of the prokaryotic to eukaryotic proteins of the caecal samples was 3:1 and these results showed that the composition of the microbiota changed among diets. Proteins belonging to *Ruminococcaceae* and *Erysipelotrichaceae* were identified in higher amount in diets containing phytase. Conversely, proteins of *Lactobacillaceae* decrease with phytase addition. Among diets with MCP addition, proteins of *Erysipelotrichaceae* and *Bacteroidaceae* were more abundant, while proteins of *Eubacteriaceae* were more abundant in diets without MCP addition.

The analyses of the rumen metaproteome suggest the application of the cheesecloth gauze filtration for the RS samples to achieve a suitable protein identification ratio (2.5:1). About 2300 prokaryotic proteins could be identified in the RS-C samples showing a distinct phylogenetic distribution compared to the 1500 prokaryotic proteins identified in the RF sample. In the latter sample proteins of *Prevotellaceae* contributed to more than 50% of the total proteins whereas in the RS sample this fraction decreased and more proteins belonging to *Lachnospiraceae* and *Ruminococcaceae* were identified.

Conclusion: Our data shows the advantage of metaproteomic datasets providing deep insights into the active fraction of the microbiota of the gastrointestinal tract and to detect possible changes regarding feeding strategies. Metaproteomic data of the broiler study highlighted the effect of dietary MCP and phytases on the composition of the chicken microbiota. The optimization of the protein extraction procedures of rumen samples was successful in improving the identification ratio of prokaryotic to eukaryotic proteins although the total number of identifications still only depicts a sparse part of the actually active microbiota. Anyhow, technical progress in mass spectrometry and an increasing availability of reference sequences enhance appropriate application of metaproteomic approaches for microbiota research in animal nutrition.

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[2] Apajalahti, J. H., Sarkilahti, L. K., Maki, B. R., Heikkinen, J. P., et al. Appl. Environ. Microbiol. 1998, 64, 4084-4088.

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123. Effects of intensity of milk replacer feeding during the pre-weaned period in calves on subsequent performance of dairy cows considering additional factors in later life

Einfluss der Intensität der Milchaustauscherfütterung von Kälbern während der Tränkeperiode auf deren spätere Leistung als Milchkühe

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Current studies have shown that intensified feeding of pre-weaning calves leads to better lactational performance in resulting dairy cows (1). The aim of the present research was to investigate the effects of feeding either conventional (C) or enhanced (E) quantities of milk replacer (MR) during the pre-weaned period in calves on performance in first and subsequent lactations of resulting dairy cows. Furthermore other factors which also might affect the lactational performance were heeded.

Methods: Performance data of 50 Holstein cows collected over several lactations were used. During first wk of life these 50 animals obtained same quantities of colostrum (6 L/d) and were subsequently randomly assigned to 2 feeding groups either receiving 24.9 kg MR (powder) over 6 wks (MRI-C) or 84.5 kg MR over 13 wks (MRI-E), respectively. MRI-C consisted of 6 L water/d with 100 g powder/L water and MRI-E was composed of 6-10 L water/d with 100-160 g powder/L water. Water and grass silage were offered *ad lib.* and calf starter (S) was supplied *ad lib.* up to 2 kg DM/d. Actual intake after 13 wks of study were 23.9 ±0.8 or 82.3 ±1.2 kg MR and 98 ±10 or 57 ±17 kg DM of S for MRI-C and -E, respectively. Average daily gain during first 15 wks of life was 783 ±73 or 906 ±81 g for MRI-C and -E, respectively. After weaning all calves were fed the same diet. From 26th wk of life until first mating with approx. 400 kg of body weight, MR groups were subdivided, either fed to achieve average daily gain (WG) of 700 or 900 g, respectively. The target age at first calving (FC) was either 24 or 36 month, respectively. The heifers reared as described before were investigated up to 4 lactations. The dairy cows obtained either a diet with normal or high mineral and vitamin contents (MVC) arranged in a 2x2 factorial design with the intensity of MR feeding (MRI-C or -E). During lactation period individually feed intake, milk yield and body weight were measured daily. Milk fat, protein and lactose content were determined twice a wk. Net energy content (MJ NEL/kg DM) of the diet components was calculated routinely. Daily fat, protein and ECM yield were computed. The ratio between milk energy production - expressed as NEL - and energy intake having accounted for maintenance was calculated to estimate the production efficiency (PE). For statistical analysis the mixed procedure of SAS® was applied.

Results: A year, LN and LP effect was observed for all investigated parameters (P<.001), whereas MRI, WG, FC, MVC, MRI*WG and MRI*FC had no significant effect. Daily protein and ECM yield and also PE were affected by MRI*LN (P<.001). MRI-C had a numerically higher milk protein yield as MRI-E in lactation 1-3, but not in 4th lactation. Whereas MRI-E was associated with a numerically higher ECM yield compared to MRI-C in lactation 1, 2 and 4, but not in 3rd lactation. Furthermore, MRI-E had a numerically better PE then MRI-C in lactation 1 and 2, but not in 3rd and 4th lactation. However, the results for MRI-C and -E within the same lactation number were not significantly different between these groups.

Conclusion: The present results did not validate a better lactational performance of dairy cows fed on an intensified level during the pre-weaning period. However, high number of influencing factors and low number of animals might have compromised the statistical power for a fully conclusive statement on possible effects of enhanced milk feeding on lactational performance.

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124. Effects of energy intake during early postnatal life of Holstein-Friesian calves on later life milk production during first lactation

Einfluss der Energieaufnahme während der frühen postnatalen Phase von Holstein-Friesian Kälbern auf die spätere Milchleistung in der ersten Laktation

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During the last years research on the effects of an increased supply of nutrients during early life in calves has been intensified. Recent studies have reported higher weight gains in combination with a decrease in abnormal behavior in intensified fed calves. The objective of this study in Holstein-Friesian calves was to assess the impact of two feeding strategies differing in nutrient supply during the first weeks of life on subsequent first-lactation milk production.

Methods: All animals were kept individually during the first week of life and in groups during the periods of rearing and first lactation. During the first 4 weeks of life, calves were fed either ad libitum (AdL; ad libitum milk feeding (17.3 MJ ME/kg DM) during week 1 and milk replacer (MR; 21.2 MJ ME/kg DM) from d 8-28; N=38) or according to a restrictive feeding protocol (RES; 4 L MR/d during week 1, 6 L MR (21.2 MJ ME/kg DM) from d 8-28; N=30). Feeding was similar in both groups after the 4th week of life. Weaning took place from week 5 to week 10 of life. All calves had free access to starter, hay and water at all times. Birth weights and weekly body weights up to the 10th week of life were recorded. After calving, milk yield during the first lactation was assessed daily and milk ingredients were measured every two weeks. Statistical analyses were performed using the MIXED procedure in SAS. In the model for feed intake and body weights group and week of life were included as fixed effects and animal as random effect. In the model for milk production traits the group, the month of lactation, the month of year were included as fixed effects, the day of lactation was included as repeated effect and the animal was considered as random effect. Bonferroni post hoc tests were performed and data are presented as LSMeans ± SEM.

Results: The average dry matter and energy intakes during the first 4 weeks were higher in the AdL-calves compared with the RES-calves (28.8 kg *vs.* 17.9 kg DM; 345 MJ ME *vs.* 286 MJ ME, respectively; $p<0.01$ each). The average starter intake did not differ between groups (0.60 *vs.* 0.76 kg/day for AdL and RES calves; $p=0.07$). In AdL-calves the average daily gain was higher compared to RES calves during the first 4 weeks of life (0.72 *vs.* 0.45 kg/day; $p<0.001$), while age at first calving did not differ between groups (765 *vs.* 777 days; $p=0.30$). In the AdL- and RES-group, 21 and 26 animals remained on the farm for a full lactation of 305 days, respectively. The average fat-corrected milk yield in the first lactation was higher in AdL-animals compared with RES-animals (29.2 ± 0.4 *vs.* 27.9 ± 0.4 kg FCM/day; $p=0.03$); concentrations of milk protein and milk fat did not differ.

Conclusion: The results indicate that an increased feeding intensity during early life has positive long-term effects on the milk production potential in the first lactation. Further research is needed to determine the effect of enhanced milk feeding in calves on subsequent life-time milk performance.

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125. Development of milk production during the colostral period and early lactation and its relation to overall lactation performance in dairy cows

Entwicklung der Milchproduktion während der Kolostralmilchphase und Frühlaktation und ihr Einfluss auf die Gesamt-Laktationsleistung bei Milchkühen

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The description of the shape of the lactation curve is commonly based on daily (after the colostral period) or weekly average milk production whereas the milk production of individual milkings immediately after parturition has not yet been investigated in terms of effects on later lactation performance. The lack of information may be due to the fact that during the colostral period milk cannot be delivered and consequently milk yield is not automatically recorded during the first few days of lactation. The objective of this study was to investigate the development of milk production during the colostral period and early lactation and its relation to overall lactation performance in dairy cows.

Methods: Seventeen primiparous and 39 multiparous (2.9 ± 0.2 parities, mean ± SEM) Holstein dairy cows (Posieux, Switzerland) were followed during the entire lactation. The milk yield of the previous standard lactations (305 d) of the multiparous cows was 8137 ± 232 kg. Cows were fed according to their energy and nutrient requirements. Multiparous cows were milked for the first time 2h and 15 min ± 15 min (range: 30 min to 5h and 15 min) and primiparous cows 4h and 50 min ± 26 min (range: 2h and 30 min to 9h and 45 min) after parturition. From the 2nd milking after parturition onwards, cows were milked twice daily at the scheduled milking times around 0500 h a.m. and 0400 h p.m. in the milking parlor. Milk yield of individual a.m. and p.m. milkings as well as daily milk yield were recorded. The development of milk yield during the first ten milkings after parturition and 28 days of lactation between multiparous and primiparous cows was compared with the MIXED procedure of SAS, version 9.2 (SAS Institute, Cary, North Carolina, USA). The model included group (primiparous or multiparous) as fixed effect. P-values < 0.05 were considered to be significant. A non-linear regression equation was used for the characterization of the lactation curve up to d 28 and its coefficients were calculated individually for each cow with SigmaPlot 11 (Systat Software, Inc., San José, California, USA). Pearson's correlation coefficients between the amount of colostrum and milk yields at different time points, time spans in-between the first 3 milkings and milk yield in the previous lactation were evaluated with the CORR procedure of SAS.

Results: Milk yield at the first milking after parturition (colostrum) ranged from 1.3 to 20.7 kg (Δ 19.4 kg) in multiparous and from 1.8 to 10.9 kg in primiparous animals (Δ 9.1 kg). At the tenth milking, milk production ranged from 9.2 to 21.5 kg (Δ 12.3 kg) in multiparous and from 7.0 to 15.2 kg (Δ 8.2 kg) in primiparous animals, respectively. Immediately after parturition, daily milk production increased rapidly, but after approximately one wk in lactation, the slope of the daily milk production curve flattened and continued more linear. A non-linear regression equation was used to determine this timely change which occurred earlier in primiparous (d 6.9 ± 0.3) than in multiparous cows (d 8.2 ± 0.2; $P < 0.01$). The correlation between the amount of first colostrum and milk production during further lactation decreased already from $r = 0.47$ ($P < 0.01$) on d 5 to $r = 0.32$ ($P < 0.05$) on d 14. In multiparous cows the correlation between total milk production of the previous 305 d standard lactation and the amount of first colostrum was not significant ($r = 0.29$; $P = 0.07$) whereas the correlation with the daily production increased from $r = 0.45$ ($P < 0.01$) on d 5 to $r = 0.69$ on d 14 ($P < 0.01$). First colostrum yield and cumulative milk production of 100, 200 and 305 lactation days were not significantly correlated in multiparous and primiparous cows.

Conclusions: Milk production during the first few milkings is widely independent from the overall production level of a cow.

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126. **Validation of an automated sensor system to count and characterize the jaw movements of cows**

Validierung eines automatisierten Sensorsystems zur Messung und Charakterisierung der Kaubewegungen von Kühen

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Unraveling the ingestive behavior of ruminants may help to better elucidate interrelations between individual animals, feed quality, intake rate, rumen health, and productive performance (1). The Rumi Watch (RW) system (Itin & Hoch GmbH, Liestal, Switzerland) comprises a noseband sensor that detects pressure changes caused by jaw movements, a data logging SD-card, and two software packages, the RW Manager that records the data, and the RW Converter that assigns the bites to four behavioral categories: eating, ruminating, drinking, and other activities. The objective of this study was to test the sensitivity and accuracy of the RW system and to validate the assignments to different activities by visual observations.

Methods: Twelve dry or lactating Holstein Friesian cows of the University of Hohenheim that received a total mixed ration were equipped with RW halters for three periods of 7 d each. After 4 d of adaptation, visually observed jaw movements and activities of cows were recorded during six separate 30-min-observation intervals per day on d 5-7, using the specially written program CowChew (CC). In four cases, sensor data could not be used due to technical problems. Thus, data from eight cows were used for comparison. For both methods (RW Converter V0.7.2.0 and CC) activities were processed on a minute-by-minute basis and summaries of observation intervals were compared by regression analysis (SAS 9.4).

Results: In total, 125 intervals covering 3860 min were compared, of which 1285, 1368, and 1207 min were assigned to rumination, eating, and other activities by visual observation, respectively. Drinking was observed during one interval only.

Table 1 Statistical parameters of the correlation of counts and minutes per activity during an observation interval recorded by the Rumi Watch sensor (RW; y) or by visual observation (CC; x) (n= 125, y= ax + b).

Model: RW V0.7.2.0 = CC	a	b	r^2	RSME	p
Total chews (n/interval)	0.99	159.0	0.94	190.7	<.0001
Ruminating chews (n/interval)	0.92	195.8	0.94	178.7	<.0001
Boli (n/interval)	0.83	3.3	0.80	5.0	<.0001
Eating chews (n/interval)	0.94	44.0	0.94	224.7	<.0001
Other chews (n/interval)	0.40	26.9	0.35	40.6	<.0001
Rumination time (min/interval)	0.90	2.8	0.95	2.5	<.0001
Eating time (min/interval)	0.85	0.2	0.95	2.4	<.0001
Other activity (min/interval)	0.96	0.0	0.96	1.9	<.0001

The RW sensor detected a greater total number of jaw movements than recorded by CC (Table 1). Resolving single jaw movements during feeding by visual observation was challenging and thus, chews may be underestimated by CC. During intervals with high proportions of other activities, RW counts were higher than those determined by CC, indicating that other head movements may produce spurious signals and lead to an overestimation of bites by the noseband sensor. The RW sensor tended to assign short intervals of 2 min or less to rumination, although cows were actually observed to be eating. This led to an overestimation of ruminating chews as well as of rumination time. Assignment of minutes to activities by RW and CC agreed by on average 91.3% when summarized results from observation intervals were considered, and by 88.4% when the corresponding values of minute-by-minute comparisons were used, which indicates that summarizing longer periods may mask differences in allocation of activities by the two methods that can only be detected at a finer temporal resolution.

Conclusions: The RW sensor proved to be sensitive and accurate to measure total number of jaw movements in cows. Nevertheless, more detailed investigations are needed to distinguish between jaw movements for feed uptake or mastication as well as to improve the converting software to be able to fully exploit the potential of the system.

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127. Hepatic mRNA expression of chemerin and its receptors and the transcriptional cofactor transducin beta-like-1 during late pregnancy and early lactation

Die hepatische mRNA-Expression von Chemerin und seiner Rezeptoren sowie des Transkriptionskofaktors Transducin beta-like-1 in der späten Trächtigkeit und frühen Laktation von Milchkühen

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During the transition period of dairy cows, fat reserves are mobilised resulting in increased nonesterified fatty acids (NEFA) concentrations in blood that cannot be fully oxidized by the liver leading to increased liver fat concentrations (LFC). Chemerin is an adipokine involved in inflammation, lipid metabolism and adipogenesis and it is highly related to obesity and insulin resistance through activation of its receptors chemokine-like receptor 1 (CMLKR1) and G protein-coupled receptor 1 (GPR1). It also participates in glucose homeostasis, where GPR1 improves glucose intolerance in obese mice (1). On the other hand, the transcriptional cofactor transducin beta-like-1 (TBL-1) is inversely correlated with LFC as its deficiency inhibits fatty acid oxidation (2). To our knowledge there is no quantitative data available on the expression of chemerin and its receptors and no data on TBL-1 in ruminant liver. We examined the mRNA abundance of chemerin, its receptors and TBL-1 during late pregnancy and early lactation in cows with different LFC.

Methods: In a previous study (3), twenty multiparous German Holstein cows (2nd to 4th lactation, >10,000kg/305d) were fed three different total mixed rations corresponding to their physiological state, i.e. far-off dry period (5.9 MJ NEL/kg DM), close-up dry period (6.5 MJ NEL/kg DM) and lactation (7.0 MJ NEL/kg DM). Liver biopsies were obtained at d −34, −17, +3, +18, and +30 relative to parturition as well as at slaughter (d +40) and samples were stored at -80 °C for further analysis. LFC was determined and ten selected cows were grouped according to their LFC at day +3 onwards in high (HLFC, LFC >30% fat in wet weight, n=5) and low (LLFC <25% fat in wet weight, n=5). The mRNAs of chemerin CMLKR1, GPR1 and TBL-1 were quantified using qPCR. Data were evaluated by repeated measures ANOVA using the MIXED model (SAS 9.3). Spearman correlations were calculated with the CORR procedure.

Results: Hepatic GPR1 mRNA expression increased after parturition ($P<0.001$) in both groups and at day +30 its abundance was higher in LLFC cows. Chemerin and CMLKR1 mRNA abundances were similar in both groups without change in time similar to TBL-1 mRNA. However, TBL-1 mRNA was less expressed ($P<0.05$) in HLFC cows at day +18. In addition, we found positive correlations between GPR1 and TBL-1 ($P<0.001$) and also for chemerin and TBL-1 ($P<0.001$), independent of LFC (HLFC: $P<0.05$; LLFC: $P<0.01$).

Conclusions: The differential mRNA expression of GPR1 with time indicates that this receptor could play an important role in liver metabolism of early lactating cows. The time course of GPR1 mRNA expression in association with chemerin mRNA abundance could be a clue for autocrine/paracrine regulation which may help to protect the liver from lipid accumulation. Moreover, CMLRK1 mRNA does not seem to have a relevant role in this process. In regard to TBL-1, our results are in accordance to the effects observed in other species, as its lower expression occurs simultaneously with increase of LFC. An increase of fatty acid oxidation linked with the activation of TBL-1 when chemerin binds to GPR1 could explain the correlation between GPR1 and TBL-1 RNA.

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128. **Influencing factors on the abundance of rumen protozoa in two *in vitro* fermentation systems**

Einflussfaktoren auf die Abundanz von Pansenprotozoen in zwei in vitro Fermentationssystemen

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Introduction: *In vitro* fermentation systems, especially continuous culture systems, are often characterised by a significant decrease of protozoa numbers in the course of incubation. This has been ascribed to a washout of protozoa due to high liquid turnover rates, to a lack of suitable sequestration area and to the exposure of fermenter contents to atmospheric oxygen. Three *in vitro* experiments were conducted to test (i) the effects of two different buffer dilution rates on protozoa numbers in Rusitec fermenters, (ii) if protozoa could be maintained in Rusitec fermenters by an enlargement of the potential sequestration area and (iii) whether or not the use of a reducing agent affects protozoa numbers after atmospheric oxygen exposure in a Hohenheim gas test (HGT).

Methods: For all experiments the rumen fluid was obtained from a rumen fistulated steer before morning feeding. In experiment 1, a Rusitec system with 3 vessels per treatment was used. The buffer dilution rates were adjusted to 0.45 d^{-1} and 0.52 d^{-1}. The nylon bags were filled with 10 g FM chopped hay (XP: 10.3 % DM, aNDFom: 52.0 % DM) and were incubated for 48 hours. In experiment 2, a Rusitec system with 4 vessels per treatment was used. To enlarge the potential protozoal sequestration area either sponge material (12 sponge cubes and a sponge disc; material: polyurethane) was added to the fermenters or an additional feed bag allowing an incubation time of 72 hours (referred to as "substrate"). The buffer dilution rate was adjusted to 0.5 d^{-1} and the feed bags were filled with 10 g FM of chopped hay and ground concentrate at a ratio of 70:30 (XP: 15.4 % DM, aNDFom: 35.5 % DM). In both experiments, samples to determine protozoa numbers were taken from the fermenter fluid immediately before the replacement of the feed bags. In experiment 3, a HGT apparatus was used with 3 syringes per treatment. Based on Rusitec inoculum, two different inocula were prepared either with or without the reducing agent Na_2S. Inocula were not purged with CO_2 during the filling procedure of the syringes, to increase oxygen exposure. The ration of hay and concentrate of experiment 2, ground to pass a 1-mm sieve, was used as substrate. For Rusitec experiments, an ANOVA was performed using a mixed model (fixed effects: treatment, incubation time; random effect: fermenter). For the HGT experiment, a two-way ANOVA was performed using a linear model (fixed effects: treatment, incubation time, treatment × incubation time). Statistical analysis was carried out using R version 3.1.0.

Results: In experiment 1, the variation of the buffer dilution rate had no significant effect on protozoa numbers in the course of incubation ($p>0.05$, Fig. 1). They decreased from initially 15 x 10^4 to 2 x 10^4 cells per mL, the strongest decrease occurring within the first day of incubation. In experiment 2, the addition of sponge material resulted in slightly but significantly lower protozoa numbers compared to the control ($p<0.01$) and substrate treatments ($p<0.001$, Fig. 2). However, protozoa numbers in fluid associated with feed bags and sponge material were 3.2 and 3.0 times higher, respectively, compared to the fermenter fluid (data not shown). Consequently, protozoa sequestration occurred in both materials. In experiment 3, protozoa numbers were reduced by about 75 % within 72 hours of incubation regardless of whether Na_2S was used or not ($p>0.05$, Fig. 3).

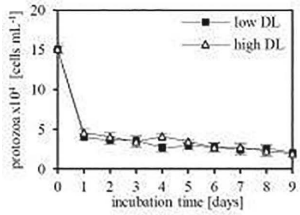

Fig. 1: Effect of dilution rate (DL) on protozoa numbers in Rusitec fermenters (mean±SD, n=3).

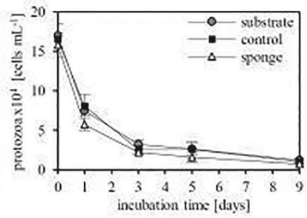

Fig. 2: Effects of added sponge material and an additional feed bag on protozoa numbers in Rusitec fermenters (mean±SD, n=4).

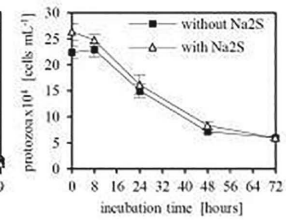

Fig. 3: Effects of a reducing agent on protozoa numbers in HGT (mean±SD, n=3).

Conclusions: Neither the reduced dilution rate nor the enlarged sequestration area maintained protozoa numbers in Rusitec fermenters at in vivo levels. Therefore, the washout is probably not the only cause for the decrease of protozoa numbers. Due to a lack of a significant effect of Na_2S, it is unlikely that the initial oxygen exposure caused the decrease of protozoa numbers in HGT.

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129. Influence of combined effect of temperature stimulation during the last days of incubation and of different protein and energy concentrations in the feed on growth performance of cockerels of different strains

Einflussfaktoren auf die Abundanz von Pansenprotozoen in zwei in vitro Fermentationssystemen

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Laying-type cockerels or spring chicken cannot be reared economically. But routine culling of these day-old male chicks is more and more an ethical problem and to find alternative solutions is a great challenge. Lohmann Tierzucht bred the dual-purpose chicken (producing eggs and meat) in response to growing criticism of conventional practices in modern egg production. Recent research with birds shows that incubation climate may have a long-lasting influence on poultry performance up to the age of slaughter. In poultry embryos at the end of incubation, peripheral and central nervous thermo-regulatory mechanisms, as well as other body functions, are well developed, so that after mild temperature variations no negative side effects will be expected. Therefore the following study was carried out, to investigate whether short-term variation in incubation temperature during the last days of incubation have a long-lasting effect on performance, also in laying-type cockerels.

Methods

2880 eggs (Lohmann Brown-LB/Lohmann Dual-LD) were incubated from days 1 to 17 under common incubation temperature (37^0C). From day 18 until hatching the eggs were sorted in hatch incubators with different temperature programs: 37^0C (control) and 1^0C over standard for 2 hours daily (38^0C: short-term warm stimulation). Chicks were sorted by sex and male cockerels were randomly distributed in 8 treatment groups (two origins of chicks-LB, LD; two hatch incubators; two different protein/energy-200 g crude protein/11 MJ AME_N/kg - low; 215 g/12 MJ - high) from day 1 to 70 of age. Data were analyzed via a three-way ANOVA (SAS).

Results and conclusion

Growing performance of LD cockerels was significantly better compared to LB males (Table 1). Final body weight of LD birds was 1000 g higher and feed to gain ratio 10% lower. Short-term temperature stimulation during the end of incubation resulted in a 3.5% higher final body weight by LD cockerels. The daily feed intake and the feed to gain ratio was significantly improved through the increased protein/energy concentration of the "high" feed.

Table 1 Feed intake, final body weight and feed to gain ratio of cockerels

Genetic line	Temperature stimulation	Feed level	Feed intake, g/ bird/day	Final body weight, g/ bird	kg feed/ kg weight gain
LB	Control	Low	47.6 43.3	1336 1360	2.568
	Control	High	47.1 44.1	1336 1374	2.293
	Stimulation	Low			2.544
	Stimulation	High			2.309
LD	Control	Low	78.6 71.4	2432 2482	2.299
	Control	High	81.9 73.9	2558 2528	2.049
	Stimulation	Low			2.275
	Stimulation	High			2.070
P-values Genetic line			<0.001 0.14	<0.001	<0.001
Temperature stimulation Feed level			<0.001	0.09 0.45	0.96 <0.001

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130. Development of a rt-PCR method for quantification of *Kazachstania slooffiae* yeast species and total yeasts occurring in the porcine gut

Entwicklung einer rt-PCR Methode zur Quantifizierung der Hefespezies Kazachstania slooffiae sowie der Gesamtzahl der im Schweinedarm vorkommenden Hefen

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Because of the scarce knowledge on yeasts isolated from the intestinal tract of pigs, alternative methods to cultivation need to be established to get more insight in this field. Therefore, the aim of the study was to develop a real time PCR (rt-PCR) protocol for the quantification of total yeasts in the porcine gut with focus on *Kazachstania* (*K.*) *slooffiae* known to dominate this environment (1).

Methods: Thirty German Landrace pigs from 11 litters were weaned at 27-28 d of age and individually caged. No antibiotics were applied before and during the entire experiment. The room temperature was set at 28 °C and lowered after 5 d to 27 °C. A diet for growing piglets and water were offered *ad libitum*. Five days after weaning, faecal samples (1 per piglet) were collected directly from the rectum and cultured on SGA medium, with 50 µg mL^{-1} chloramphenicol, for 5 d at 37 °C (1). The colony forming units (CFU) were given as log CFU g^{-1} faeces. Genomic DNA (gDNA) were extracted from pure cultures of yeast occurring in the piglets' gut (*Issatchenkia orientalis, Geotrichum candidum*, *Rhodotorula mucilaginosa, Pichia guilliermondii*, *Trichosporon asahii*, *K. telluris*, *K. slooffiae;* and *Candida* (*C.*) spp: *C. parapsilosis*, *C. glabrata*, *C. catenulata*, *C. tropicalis*) using FastDNA® SPIN Kit, and from faeces using PowerLyzerTM PowerSoil® DNA Isolation Kit according to manufacturers' protocols. DNA concentration was measured using Quant-iT™ dsDNA Broad-Range Assay Kit on a Qubit® 1.0 Fluorometer. Primers NL1/ LS2 (for 26S rDNA) were applied to quantify the number of total yeasts. For quantification of *K. slooffiae*, 2 new primer sets, KS-f/KS-r (for 26S rDNA) and KSact-f/KSact-r (for *act1* gene), were designed by means of Primer-Blast tool (2). gDNA of *K. slooffiae* was used as a calibrator for quantification of *K. slooffiae* and total yeasts. All samples were analyzed in triplicate on a StepOnePlus Real-Time PCR System (v. 2.2) using MyTaqTM HS Mix followed by melt curve analysis. Optimal annealing temperature (Ta) for primers was determined. The rt-PCR was performed using one-point calibration (OPC) method (3). To determine the effect of different Ta of NL1/LS2 primers on the qPCR performance, the values of individual amplification efficiency (E) and the means of log copy numbers were analyzed by one-way ANOVA. Pearson correlation coefficient was calculated between log CFU and log copies. All procedures were performed using IBM SPSS Statistics for Windows (v. 20.0). The level of significance was pre-set at $P < 0.05$.

Results: Copies in gDNA can be determined by rt-PCR using PCR amplicons as a calibrator and OPC method. The values of quantitation cycle and E of gDNA calibrator were highly reproducible. The qPCR results using primers NL1/LS2 correlated ($r = 0.984$, $P < 0.0001$) with cultivation results. From two primer sets developed, KSact-f/KSact-r was suitable for quantification of *K. slooffiae*. The copy numbers of *K. slooffiae* could be determined in 40% of analysed animals, amounting to about 70% of total yeasts.

Conclusion: The results of the present study shown that the rt-PCR protocol can be successfully used for the quantification of total yeasts and *K. slooffiae* in the porcine gut. The application of this method in following studies will help to understand more about these microbes in the gut of pigs.

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131. Effects of general anesthesia with ketamine and neuroleptic sedatives on plasma metabolites and hormones in pigs

Wirkungen einer Vollnarkose mit Ketamin und Muskelrelaxans auf Metaboliten und Hormone im Plasma beim Schwein

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Plasma metabolites are often determined during or after general anesthesia (GA). However, there is no concise information on whether and how GA influences metabolite concentrations. Ketamine is commonly used for GA in swine. When used alone, it causes strong excitations and thus a relaxant anesthetic/sedative is co-applied. We investigated whether plasma metabolites and hormones are influenced by GA induced with ketamine (K) and two different sedatives, namely azaperone (A) and xylazine (X).

Material and methods: In two experimental repetitions, 6 female pigs (147 ± 7.5 kg) fitted with jugular vein catheters were used. On the first experimental day (ED) basal measurements were made on plasma samples collected at regular intervals for a period of 6 h (CON). On the following EDs, each pig was anaesthetized with a single administration of A (3 ml Stresnil®) or X (5 ml Xylazin 2% Bernburg®) on two different days, separated with a one-day-washout period (1d-WP). Following another 1d-WP, the pigs received each of the relaxants in combination with K (20 ml Ursotamin®; AK or XK) on two different EDs, separated with a third 1d-WP. Plasma concentrations of glucose, lactate, NEFA, triglycerides (TG), 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-hocs with the Tukey-Kramer test.

Results: All treatments induced GA. Except for glucagon, all metabolites and hormones were affected ($P<0.05$) by GA. Glucose concentration was increased ($P<0.05$) by A and X with the latter exerting a stronger effect ($P<0.05$). Effect of XK on glucose was comparable to that of AK ($P>0.05$), but higher ($P<0.05$) than that of A alone. Time dependent effects of AK and XK indicated that glucose concentration was more rapidly increased due to X than A and the increase lasted longer. Lactate concentration was elevated ($P<0.05$) by A, and ketamine (AK) intensified this effect ($P<0.05$). Administration of X alone and as XK did not influence ($P>0.05$) lactate level, and prevented the ketamine-associated increase when administered as XK. Plasma NEFA concentrations were increased by A alone and in combination with ketamine (AK). Inclusion of ketamine did not further increase NEFA concentrations. Plasma TG concentrations were elevated by A and AK, however remained unaffected by X in either form (X, XK). Ketamine did not influence TG concentrations. Cholesterol was increased ($P<0.05$) only by the combination treatment AK. Insulin concentration was reduced only by X ($P<0.05$) administered either singly or as XK. Cortisol concentrations were elevated by A, and even more so by AK ($P<0.05$), while it showed no change ($P>0.05$) with X.

Conclusions: With the exception of glucose, A and AK strongly increased plasma levels of all the metabolites, likely mediated through the GA effects on cortisol. From the two relaxant sedatives, X induced fewer changes than A, thus it may be suggested for GA with K.

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132. Investigations on sulfur and sulfate contents in roughages and concentrates for ruminants

Untersuchungen zum Schwefel- und Sulfatgehalt in Grund- und Kraftfuttermitteln für Rinder

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Sulfur has been a "forgotten" mineral in animal nutrition for a long time; feedstuffs contained enough sulfur (or S-amino acids, resp.). Already decades ago different feeds were analysed regarding the S-content (1,2), but the SO_4^{2-} content was tested very seldom or sporadically only (for example by 3). Meanwhile, interest in sulfur in plant nutrition increased (fertilisation of field crops and grassland!). From the veterinary point of view types and amounts of ingested sulfur in animals have deserved interest (H_2S-poisoning and DCAB-concept (4), SO_4^{2-} as a laxative agent in milk replacers; 5). The present study is part of a project about etiological clarification of severe diarrhea in dairy farms of unknown reason. Against this background focus was put on roughages but also on further potential sulfate sources (including concentrates).

Methods: Different feedstuffs for ruminants (see table 1) were investigated, whereby most of them were sent to the institute for different reasons (impaired health/reduced performance) from various regions of Germany (mainly northwest parts). Up to now detailed information regarding site, soil, fertilizing or use of S-containing compounds/additives are missing, thus a statistical evaluation does not make sense at this stage of the project. Sulfur was analysed by *Dumas* (*Vario CNS*, elementar®). Sulfate contents were determined with gravimetric method (precipitation with barium chloride).

Results: There was a large variation in the sulfur and sulfate contents between and within the groups of feedstuffs but also feed specific peculiarities were observed. In grass silages increasing sulfur and sulfate contents from the 1. to the 4. cut were found, a similar tendency was obvious in grass. The contents in corn silage were generally lower and showed only slight variations. S from SO_4^{2-} contributed between 23 and 61% of total S.

Table 1: Sulfur and sulfate contents in various feedstuffs for ruminants

		S (g/kg DM)			SO_4^{2-} (g/kg DM)			SO_4^{2-}-S, %
feedstuffs	n	mean ± SD	min	max	mean ± SD	min	max	total S
grass / pasture 1. cut	25	2.28 ± 0.77	1.15	4.44	3.13 ± 1.94	0.94	8.68	41
following cuts	17	4.49 ± 1.90	2.08	8.97	7.90 ± 4.91	2.04	18.0	58
grass silage								
1. cut	42	2.98 ± 0.50	2.23	4.16	5.11 ± 1.36	2.37	8.16	57
2. cut	33	3.05 ± 0.63	1.97	4.35	5.36 ± 1.84	1.39	8.39	56
3. cut	12	3.60 ± 0.63	2.35	4.58	6.46 ± 1.65	4.56	9.36	55
4. cut	7	5.67 ± 0.86	2.52	4.75	7.53 ± 1.57	5.27	10.1	61
hay	23/32[1]	2.17 ± 0.66	1.18	4.17	5.04 ± 2.28	1.83	8.97	- [2]
alfalfa (dried)	11	2.54 ± 0.89	1.43	4.22	4.49 ± 1.89	2.62	8.03	58
corn silage (whole plant)	13	1.16 ± 0.18	0.75	1.49	0.81 ± 0.27	0.31	1.13	23
soy bean meal	9	4.35 ± 0.38	3.78	4.84	4.30 ± 1.37	1.89	5.87	29
rapeseed meal	12	7.16 ± 0.33	6.63	7.59	6.46 ± 1.21	3.97	8.76	30
dairy concentrates	10	3.53 ± 0.75	2.37	4.50	5.02 ± 1.20	3.60	7.52	48
$TMR_{total / partly}$	5/6[1]	2.83 ± 0.30	2.48	3.32	4.87 ± 1.99	3.84	8.92	- [2]

[1] samples (n) analysed for S / SO_4^{2-}; [2] either S or SO_4^{2-} analysed

Conclusion: In grass fodder up to 60% of total S derived from sulfate, in most grass silages more than 50%. A sulfate content that might result in risks of inducing diarrhea due to laxative effects of sulfates was not an exception. Besides of the type of feed, sulfur and sulfate contents in feeds depend on soil, fertilisation and the date of harvesting. As described before diarrhea symptoms arise in calves with sulfate concentrations from 6 g/kg milk replacer powder (5). In adult cattle (~500 kg) an intake of 100 up to 250 g might result in diarrhea (6). The unexpected high SO_4^{2-} content of some concentrates such as rapeseed meal might contribute additionally to the sulfate load in cattle.

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133. Laboratory investigations to characterise the dietary phosphorus in compound feeds for pigs and poultry by its solubility in water and dilute HCl with and without incubation

Laboruntersuchungen zur Charakterisierung des Phosphors im Mischfutter für Schweine und Geflügel anhand seiner Löslichkeit in Wasser und verdünnter HCl mit und ohne Inkubation

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Introduction: Although phosphorus (P) requirements of monogastric species are nowadays mostly formulated as "digestible P" or "non-phytate P", routine laboratory analyses only determine the total P content of diets without any specification (P sources, interactions, availability), but in vitro methods to characterise dietary P are under development (1). The aim of this study was to evaluate the P-solubility in water and an acid medium by laboratory analyses when a test diet was supplemented by different mineral P sources and $CaCO_3$.

Material and methods: Monocalciumphosphate (MCP), dicalciumphosphate (DCP), monodicalciumphosphate (MDCP), and calciumsodiumphosphate - also named defluorinated phosphate (DFP) - were added to a "test diet" based on wheat, barley, soybean meal and rapeseed meal in such way that a total P content of ca. 6 g/kg (with Ca contents between 3.1 and 5.2 g/kg, depending on the supplemented feed phosphate) was reached with a constant proportion of 96 % test diet in all supplemented diets. Each diet was analysed for P solubility in water and 0.4 % HCl after 1 min of shaking as well as after 90 min of incubation with stirring and following centrifugation. The contents of soluble P were determined only in the *supernatants* by the molybdate-vanadate-method after ashing in the microwave digestion unit. To evaluate the influence of higher Ca contents on the P solubility the procedure was repeated with additional supplementation with $CaCO_3$ so that every diet reached a Ca content of ca. 14 g/kg. Statistical analyses were done by using Fisher's LSD-test (GLM-procedure) and two-sample t-test of SAS®.

Results: In this model test diet the supplementation of different feed phosphates led to different contents of soluble P as shown in the table (mean ± s.d.; n = 3).

Diet-type (n=3)	Total P (g/kg)	Soluble P (g/kg)			
		H_2O + 1 min	H_2O + 90 min	HCl + 1 min	HCl + 90 min
Test diet[1)]	$3.97^{bA} \pm 0.08$	$1.23^{cA} \pm 0.14$	$2.54^{cA} \pm 0.04$	$1.21^{cB} \pm 0.06$	$1.39^{cB} \pm 0.11$
+ MCP	$5.91^{aA} \pm 0.12$	$2.61^{aA} \pm 0.07$	$3.66^{aA} \pm 0.09$	$3.14^{aB} \pm 0.14$	$3.49^{abB} \pm 0.25$
+ MDCP	$5.94^{aA} \pm 0.13$	$1.88^{bA} \pm 0.16$	$3.15^{bA} \pm 0.10$	$3.35^{aA} \pm 0.19$	$3.60^{aB} \pm 0.02$
+ DCP	$6.01^{aA} \pm 0.08$	$1.30^{cA} \pm 0.12$	$2.52^{cA} \pm 0.07$	$3.27^{aA} \pm 0.21$	$3.40^{abB} \pm 0.30$
+ DFP	$5.91^{aA} \pm 0.23$	$1.21^{cA} \pm 0.04$	$2.32^{dA} \pm 0.12$	$2.21^{bA} \pm 0.10$	$3.22^{bA} \pm 0.23$
Test diet + $CaCO_3$[2)]	$4.13^{bA} \pm 0.13$	$0.99^{cA} \pm 0.08$	$1.91^{dB} \pm 0.13$	$1.70^{dA} \pm 0.13$	$2.37^{dA} \pm 0.10$
+ MCP	$5.93^{aA} \pm 0.29$	$2.57^{aA} \pm 0.04$	$3.46^{aA} \pm 0.29$	$3.68^{aA} \pm 0.13$	$4.42^{aA} \pm 0.17$
+ MDCP	$5.97^{aA} \pm 0.08$	$1.79^{bA} \pm 0.14$	$2.81^{bB} \pm 0.09$	$3.35^{abA} \pm 0.05$	$4.12^{bA} \pm 0.06$
+ DCP	$5.96^{aA} \pm 0.14$	$1.11^{cA} \pm 0.22$	$2.24^{cA} \pm 0.17$	$3.23^{bA} \pm 0.25$	$4.23^{abA} \pm 0.18$
+ DFP	$6.09^{aA} \pm 0.04$	$1.11^{cA} \pm 0.12$	$2.07^{cdB} \pm 0.07$	$2.33^{cA} \pm 0.31$	$3.27^{cA} \pm 0.11$

[1)] calculated on 96 % according to its proportion in the following compound diets [2)] in total: ca. 14 g Ca/kg

[a, b, c, d] indicate significant differences of diet-types ($p < 0.05$) within both groups with and without $CaCO_3$

[A, B] indicate significant differences of corresponding diets with and without $CaCO_3$ ($p < 0.05$)

In the diets without $CaCO_3$ the water solubility was highest after supplementation with MCP, followed by the diet containing MDCP. The contents of HCl-soluble P after 90 min of incubation were higher in all supplemented diets compared to the test diet without feed phosphate. After 1 min in HCl the content of soluble P in the diet with DFP was lower than in those ones containing MCP, MDCP and DCP. Adding the $CaCO_3$ reduces the water solubility in the test diet and had smaller but for MDCP and DFP still significant effects on the contents of water-soluble P after 90 min in the supplemented diets. The HCl solubility after 90 min of incubation was higher in all diets except the one containing DFP, where the values were similar to the diet without $CaCO_3$. These increases might be caused by the buffering effect of $CaCO_3$ on the very acidic medium which facilitates the enzymatic hydrolysis (native phytase of cereals) of phosphate during the incubation.

Conclusion: In a test diet based on cereals and protein sources the supplementation of different feed phosphates led to a different P-solubility, related to the particular mineral P compounds (2). But the contents of soluble P also seem to depend on further factors like Ca-contents, pH and buffering capacity. Presumably the soluble P levels found here after 90 min in a strong acid represent more P than is digested/absorbable in vivo.

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134. Acute and subacute response of iron, zinc, copper and selenium in pigs experimentally infected with *Actinobacillus pleuropneumoniae*

Eisen-, Zink-, Kupfer- und Selen-Reaktionen bei experimenteller Actinobacillus pleuropneumoniae Infektion bei Schweinen

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The model of experimentally induced porcine pleuropleumonia by aerosolic infection with the Gram-negative bacterium *Actinobacillus pleuropneumoniae* (*A.pp*.) was previously used to study biomarkers or serological, bacteriological or immunological issues. Trace elements such as copper (Cu), iron (Fe), zinc (Zn), and selenium (Se) were also suspected to be involved in the acute phase reaction (APR), most likely in coherence with known binding proteins in serum. This study was performed to characterise the response of Fe, Zn, Cu and Se in *A.pp*.-induced porcine APR (1).

Methods: Twenty piglets were challenged by aerosolic infection with *A.pp.* serotype 2, ten piglets serving as controls. Blood sampling was done initially and at day 4 and 21 after infection. Collection of liver tissue was done at day 21 (autopsy). Twice a day commercial feed was supplied. The analysis of trace elements in food resulted in the following mean concentrations (wet weight, ww): 152 mg Cu/kg ww, 123 mg Zn/ kg ww, 299 mg Fe/kg ww, 0.45 mg Se/kg ww. Hematologic parameters, serum protein fractions, APR-related proteins in serum, glutathione peroxidase and trace elements (serum/plasma and liver tissue) were analysed.

Results: The experimental *A.pp.*-infection caused fever and respiratory symptoms. APR at day 4 after infection was marked by an increase in total white blood cells, granulocytes and monocytes in whole blood samples and an increase in globulin/albumin ratio (G/A), α2-globulins, C-reactive protein, haptoglobin, ceruloplasmin (Cp), Cu and Se in serum. Concurrently, there was a decrease in haemoglobin (Hb) and packed cell volume (PCV) in whole blood as well as a decrease in albumin, transferrin, total iron binding capacity and Fe in serum and Zn in plasma. The subacute stage at day 21 was characterised by progressively increased concentrations of lymphocytes, G/A, ß-globulins and γ-globulins reflecting the specific immune reaction. Hb and PCV showed further decreases, all other parameters returned to the initial concentrations. Glutathione peroxidase activity in plasma and liver tissue remained unaffected by *A.pp*.-infection. The liver concentration (day 21) of Zn was found to be higher, that of Se was lower in the *A.pp*.-group, whereas hepatic concentrations of Cu and Fe were not affected by *A.pp*.-infection compared to the controls.

Conclusions: In summary, the model of *A.pp*.-infection proved advantageous for evaluating trace element response showing a distinct impact and the individual effects of bacteria-induced APR on Fe, Zn, Cu and Se metabolism. Se was only marginally affected by the *A.pp*.-infection. The elevated serum Cu concentration may be a side effect of the transient hepatic induction of Cp synthesis. Zn responded, being distinctly reduced in plasma and probably having been sequestered in the intracellular compartment as shown in the liver tissue. Reduction in serum Fe can be regarded as an unspecific hepcidin-regulated defense mechanism in *A.pp*-infection, presumably to withdraw Fe from bacterial acquisition systems. Additionally, the results underline that blood and liver samples of acute and subacute diseased animals are unsuitable for assessing the current nutritional trace element status of the herd and may cause misleading interpretations.

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135. Oxidative status of fattening pigs fed diets high in inorganic selenium and manganese

Oxidativer Status von Mastschweinen, die mit hohen anorganischen Selen- und Mangankonzentrationen gefüttert wurden

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In a feeding trial with fattening pigs the impact of the recommendation and EU-upper level supplementation of inorganic Se and Mn on antioxidative enzyme activity and oxidative stability in liver, muscle and plasma should be tested. Data are scarce regarding combined high Se and Mn supplementation, however, as Se and Mn are functionally related as cofactors of glutathione peroxidase (GPx) and Mn-superoxide dismutase (SOD), respectively, beneficial synergistic effects may result.

Methods: 60 female fattening pigs ((Large White*German Landrace)*Piétrain) were assigned to four treatments (three replicates). In a 2x2-factorial approach Se and Mn were fed in two levels (0.2 vs. 0.5 mg Se/kg DM as Na-selenite; 20 vs. 150 mg Mn/kg DM, as MnO) during fattening (32.4±0.3kg to 116.0±0.2kg body weight). Grower (13.4MJ ME/kg, 16.4% CP) and finisher (13.5MJ ME/kg, 14.3% CP) diets based on barley, maize and soybean meal were provided ad libitum in meal form. Liver (left lobe), abdominal muscle tissue and plasma obtained at slaughter were analysed for antioxidative enzyme activities (GPx, SOD, catalase (CAT)), thiobarbituric acid reactive substances (TBARS) and total antioxidative capacity (TAC, CUPRAC assay). Two-way-ANOVA (SAS 9.4) with experimental factors (Se, Mn), interaction (Se*Mn) and replicates in the model was computed (alpha=0.05).

Results: Overall performance was unaffected by treatment ($p>0.10$), however, Mn reduced ADG and increased FCR in grower diet ($p<0.05$). Activity of GPx in liver was increased due to high Se diets ($p<0.05$). Mn supplementation increased CAT activity in liver, GPx in plasma and TAC concentration in muscle, whereas it decreased CAT activity in plasma ($p<0.05$). A significant interaction for Cu/Zn-SOD in muscle showed higher activity in single high Se-diets but lower activity combined with Mn-high supplementation. TBARS concentrations remained unchanged with mean values of 142.2±1.5 mg/kg in liver and 19.8±0.45 mg/kg in muscle ($p>0.10$).

Table: Oxidative status of liver, muscle and plasma in fattening pigs fed high inorganic Se and Mn

	Treatment					p-value[1]		
	Control	Se	Mn	Se+Mn	SEM	Se	Mn	Se*Mn
Liver GPx, U/g protein	204.3	234.8	215.3	220.7	4.6	*	n.s.	n.s.
Liver SOD, U/mg protein	11.96	12.93	12.23	12.75	0.37	n.s.	n.s.	n.s.
Liver Mn-SOD, U/mg protein	2.67	2.56	2.63	2.65	0.08	n.s.	n.s.	n.s.
Liver Cu/Zn-SOD, U/mg protein	9.27	10.37	9.61	10.14	0.34	n.s.	n.s.	n.s.
Liver CAT, U/mg protein	892.2	912.6	986.3	976.6	16.2	n.s.	*	n.s.
Liver TAC, mg AEQ/g	10.51	10.59	10.73	10.81	0.08	n.s.	n.s.	n.s.
Muscle GPx, U/g protein	12.46	12.77	10.91	13.31	0.42	n.s.	n.s.	n.s.
Muscle SOD, U/mg protein	1.33	1.34	1.23	1.27	0.03	n.s.	n.s.	n.s.
Muscle Mn-SOD, U/mg protein	1.03	0.95	0.92	0.97	0.03	n.s.	n.s.	n.s.
Muscle Cu/Zn-SOD, U/mg protein	0.30^{ab}	0.39^{a}	0.35^{ab}	0.26^{b}	0.02	n.s.	n.s.	**
Muscle CAT, U/mg protein	6.37	6.28	6.14	6.27	0.12	n.s.	n.s.	n.s.
Muscle TAC, mg AEQ/g	3.95	4.02	4.11	4.10	0.03	n.s.	*	n.s.
Plasma GPx, U/mL	1.03	0.98	1.13	1.16	0.03	n.s.	*	n.s.
Plasma CAT, U/mL	190.1	195.4	152.7	148.8	4.9	n.s.	**	n.s.

[1] ** = p0.10

Conclusions: Feeding inorganic Se well above recommendation levels could still increase liver GPx activity. While no further activity increase of MnSOD was observed in Mn groups, high Mn diets showed interactions with Fe dependent antioxidative defense. However, no synergistic effects of high Se and Mn diets or an overall beneficial impact on lipid peroxide status could be observed.

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136. Effect of milling methods, thermal treatment, and particle size of feed in layers on prececal and total tract digestibility as well as on trace element content of eggs

Einfluss der Vermahlung, Hitzebehandlung und Partikelgröße des Futters auf die Verdaulichkeit sowie die Retention von Spurenelementen im Ei von Legehennen

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Introduction: Trace elements are important for biochemical processes in body as well as egg formation. For instance, zinc status of hen is directly related to activity of carbonic anhydrase (a calcium binding protein), which is essential for egg shell formation (1). Copper deficiency in the laying hen resulted in eggs with abnormal size and shape, wrinkled and rough textured shells, and an increase in shell-less eggs (2). The inadequate manganese and zinc contents in the diet resulted in impaired shell strength (3). The effects of feed form, thermal treatment and particle size on poultry performance are well documented, however these effects on trace elements digestibility have not been tested in detail. Therefore, the present study investigated the impact of roller (R) and hammer (H) mills, mash (M) and expandate (E) with fine (F) and coarse (C) particle sizes in laying hens, on apparent ileal absorption (AIA), apparent total digestibility (ATD) and retention of zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe), in yolk, albumen and shell. **Methods:** A total of 384 hens (Lohmann Brown), 19 weeks old, were assigned using a randomized design with a 2×2×2 factorial arrangement. Feed was supplemented with mineral and vitamin premix (Spezialfutter Neuruppin, Neuruppin, Germany). The diet contained 0.078, 0.102, 0.018 and 0.534 g/kg zinc, manganese, copper and iron respectively, on dry matter basis as analyzed. Eight experimental diets were offered ad libitum during the whole experimental period and one week before for diet adaption. Ileal and rectal digesta samples (individual) as well as eggs were collected and then pooled at age of 23 weeks. The digestibility of the trace elements was determined using titanium dioxide as indigestible marker at 2 g/kg diet. The trace elements concentrations in feed, ileal and rectal digesta, yolk, albumen and shell were determined by atomic absorption spectrometry (AAS). For statistical analysis variables were subjected to ANOVA using the GLM procedure of SPSS 20.0. **Results:** The AIA of Zn, Cu and Fe was higher in treatment R in comparison with treatment H ($P < 0.05$). The ATD of Cu and Fe was higher in treatment R than treatment H ($P < 0.05$). The ATD of Fe was higher in treatment C in comparison with treatment F ($P<0.05$). The Cu concentration in yolk was higher in treatment C than treatment F ($P<0.05$). The concentration of albumen Mn was higher in treatment M as compared to treatment E ($P\leq0.04$). The particle size affected albumen concentrations of Cu which was higher in treatment C than treatment F ($P\leq0.03$). A few interaction effects between treatments were observed where milling method and thermal treatment had a significant interaction effect on AIA and ATD of Mn and Cu ($P<0.05$). The ATD of Fe and albumen Zn concentration were significantly affected by interaction between thermal treatment and particle size ($P<0.05$). **Conclusions:** Feed produced by hammer mill had negative effect on AIA and ATD for Zn, Cu, Mn and Fe, which might influence other biochemical processes in hen body; however trace elements concentrations were mostly comparable for all treatments in egg contents. Therefore, milling methods, thermal treatment and particle sizes used in present study may be used for layer feed formulation without negatively affecting egg quality.

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137. Negative Calcium balance in adult dogs after feeding calcium minimum requirements

Fehlende Effizienzsteigerung der quantitativen intestinalen Ca-Absorption nach Fütterung von Rationen mit niedrigem Ca-Gehalt bei Hunden

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Introduction: A recent meta-analysis of calcium (Ca) digestibility in adult dogs suggests that dogs do not adapt their faecal Ca excretion or apparent Ca digestibility to a low Ca intake[(1)]. Data used for this meta-analysis were mainly from short term studies with an experimental period of 4 weeks. The resulting hypothesis was that, either dogs do not adapt efficiently to a low Ca intake by increasing Ca absorption from the gastrointestinal tract or that adaptation takes longer than 4 weeks. The present study was carried out to assess if the adult dog will increase efficiency of intestinal Ca absorption when challenged for a longer period of 28 weeks by feeding diet containing the NRC minimum requirement of available Ca [(2)].

Materials and Methods: Six female Beagles (Body weight 14.5±1.5 kg) and 5 female Foxhound-Boxer-Ingelheimlabradors (FBI) (BW 25.7±1 kg), aged 3-6 years, were fed a customised diet that met Ca at minimum requirements[(2)] with a Ca:P ratio of 0.6. The Ca source was Ca carbonate, the main ingredients were grains and animal fats. The phytate content was 0.08%. Digestion trials (5-day faecal collection) were carried out in weeks 7, 14, 21 and 28 of the study. Samples were analysed for Ca content byflame-emission photometry. During every digestion trial blood samples were taken. The osteoclastic bone marker serum crosslaps was measured by an ELISA. Data wereanalysed by one way ANOVA with repeated measurements and are presented as mean ± SD.

Results: All dogs remained healthy throughout the study. The apparent Ca digestibility was negative throughout the whole study. There was no significant increase ($p>0.05$) of apparent Ca digestibility comparing the first and the last trial. In addition, there was just a significant decrease of faecal Ca excretion from the first to the second digestion trial ($p=0.032$), but not when comparing the other trials (table 1). The osteoclastic bone marker serum crosslaps increased during the study significantly ($p<0.001$). There was no effect of breed.

Table 1: Mean of Ca intake, faecal Ca excretion, apparent Ca digestibility and serum crosslaps

	Ca intake mg/kg $BW^{0.75}$	Faecal Ca excretion mg/kg $BW^{0.75}$	Apparent Ca digestibility %	Serum Crosslaps ng/ml
Week 7	60 ± 3	134± 48	-123 ± 75	0,13 ± 0,05
Week 14	61 ± 3	100 ± 24	-65 ± 35	0,18 ± 0,09
Week 21	59 ± 2	118 ± 31	-99 ± 55	0,26 ± 0,09
Week 28	57 ± 1	108 ± 33	-88 ± 60	0,28 ± 0,10

Conclusion: Adult dogs fed Ca in amounts approximately matching the minimum requirements showed a strongly negative apparent Ca digestibility. The dogs did not increase their Ca absorption efficiently enough to come close to Ca equilibrium under these conditions. The serum crosslaps suggest they used their bone Ca stores to maintain their Ca homoeostasis. A similar observation was made in sheep[3)] suggesting species differences in the relative importance of intestinal absorption and resorption for maintaining Ca homeostasis

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138. Effects of short-term reduction in alimentary zinc supply on zinc distribution in various tissue fractions of weaned piglets

Zum Einfluss einer kurzfristigen Reduzierung der alimentären Zinkversorgung auf die Zinkverteilung in verschiedenen Gewebefraktionen von Absetzferkeln

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Question: Current data on the effects of insufficient alimentary zinc (Zn) supply on tissue zinc distribution is mainly based on experiments using severely Zn deficient animal models. Therefore, the present investigation aimed in evaluating the effects of short-term undersupply of Zn on tissue Zn distribution in weaned piglets.

Material and Methods: The experiment was conducted according to [1]. A common piglet diet mainly composed of corn and soybean extracts was added with 8 levels of Zn sulfate resulting in total dietary Zn of 28 (native Zn), 34, 39, 43, 48, 58, 68, and 88 mg/kg. 48 weaned piglets were fed uniformly the diet with 88 mg/kg for 14 days and were then switched to one of the modified diets for 8 days (n=6). Animals were sacrificed and tissues were dissected for determination of Zn. Data analysis included two-way ANOVA (feed zinc, litter).

Results and Discussion

Feed zinc content	mg/kg	28	34	39	43	48	58	68	88	[1]SEM	p-value
Tissue Zn content											
Liver[3)]	mg/kg DM	83^{cd}	87^{cd}	77^{d}	88^{cd}	89^{cd}	95^{cb}	106^{b}	127^{a}	8.13	<0.0001
Blood plasma[3)]	mg/L	0.21^{d}	0.32^{c}	0.29^{dc}	0.34^{c}	0.34^{c}	0.47^{b}	0.45^{b}	0.63^{a}	0.03	<0.0001
Femur[3)]	mg/kg ash	180^{d}	198^{dc}	197^{dc}	190^{dc}	208^{bc}	210^{bc}	229^{ba}	239^{a}	6.54	<0.0001
Jejunum	mg/kg DM	76^{ba}	72^{ba}	73^{ba}	71^{b}	79^{ba}	75^{ba}	79^{ba}	81^{a}	2.37	0.02
Pancreas	mg/kg DM	76^{ba}	79^{ba}	70^{b}	71^{b}	79^{ba}	82^{ba}	86^{ba}	91^{a}	4.00	0.005
Kidney	mg/kg DM	59^{b}	64^{b}	67^{ba}	60^{b}	65^{ba}	67^{ba}	67^{ba}	77^{a}	3.23	0.01
Heart	mg/kg DM	62^{a}	60^{a}	56^{ba}	51^{ba}	59^{a}	56^{ba}	60^{a}	63^{a}	2.25	0.004

Note: [1]SEM=standard error of means; [2]$p \leq 0.05$ was considered as indicator of significant effects; [3]Data obtained from [1]

Liver Zn first responded to decreasing dietary Zn with linear reductions but exhibited a sharp turnaround towards a plateau at ca. 60 mg/kg of dietary Zn. This denotes the transition from sufficient to deficient dietary Zn supply [1, 2].

Zn in blood plasma, femur, jejunum, pancreas and kidney showed a linear decrease over the entire range of dietary Zn. This agrees to long-term zinc deficiency studies in case of Zn undersupply [e.g. 3]. But at sufficient supplies, Zn contents of these tissues usually exhibit a plateau. Mobilization of Zn from bone stores observed in the present study even at long-term sufficient dietary Zn may be explained by the lag-time in homeostatic adaptation of Zn metabolism. Obviously, any reduction of dietary Zn supply from constant feeding conditions induces a short-term Zn deficiency. This may explain why also heart and kidney Zn did not remain constant as it is reported for the situation of long-term Zn deficiency. Heart showed a curvilinear response with Zn levels rising to initial values at lowest dietary Zn.

Conclusion: Reductions in dietary Zn may induce transient undersupplies to the entire organism irrespective of the long-term supply status (deficient vs. sufficient). The heart seems to be a highly privileged tissue for quick restoration of proper Zn contents, obviously due to its significant role for acute survival.

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139. Effects of different dietary levels of phosphorus and calcium on the mitogen-induced proliferation of mesenteric lymph node lymphocytes, the intestinal microbiota and microbial activity in pigs

Einfluss unterschiedlicher diätetischer Phosphorkonzentrationen auf die mitogen-induzierte Proliferation von Lymphozyten der mesenterischen Lymphknoten, die intestinale Mikrobiota und die mikrobielle Aktivität beim Schwein

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Phosphorus (P) is a non-renewable resource and an essential nutrient both for the gastrointestinal microbial ecosystem and the host. There is little information on the impact of dietary P on the immune system of pigs, whereas several studies have shown interactions between dietary calcium-phosphorus (CaP) supply, fermentation activity and microbial composition of the gastrointestinal tract in pigs (1). The present study was designed to examine the impact of using 2 different protein sources and 2 different CaP levels on gut microbiota, microbial activity and the intestinal immune system in the pig.

Methods: Growing pigs (N=31, initial body weight 54.7 kg ± 4.10 kg) were used in 2 consecutive experiments and were allotted to 4 treatments, either fed a corn-soybean meal or a corn-pea meal-based diet, each with two different CaP levels: low (80% of requirement, Ca 3.8-4.0 g per kg of diet, P 3.6-3.7 g per kg of diet) vs. high (120% of requirement, supplemented with monocalcium phosphate, Ca 7.4 g per kg of diet, P 6.5-6.7 g per kg of diet) for 8 weeks. After slaughtering, digesta was collected to determine caecal ammonia concentration and jejunal and caecal 16S rRNA gene copy numbers of total eubacteria, and the mainly proteolytic bacteria *Enterobacteriaceae* and *Bacteroides-Prevotella-Porphyromonas* by use of quantitative real-time PCR. Jejunal and ileal mesenteric lymph nodes were sampled to assess the immune cell functionality by the mitogen-induced response of lymphocytes against concanavalin A (ConA) and pokeweed mitogen (PWM). Statistical analyses were performed using the statistical package R version 3.1.0 (2). Treatment effects and their interactions were analyzed using linear mixed-effect models with the function "lmer" of the R package lme4 (3). For every model, residuals were checked for normal distribution by the Shapiro-Wilk test and variance of homogeneity by a visual check of a plot of the fitted values against the residuals. To achieve normal distribution, logarithmic or square route transformation was used for those parameters that were not normally distributed or where variance homogeneity could not be assumed.

Results: There were no interactions between CaP levels and protein sources. Gene copy numbers of total eubacteria ($P < 0.01$), *Enterobacteriaceae* ($P < 0.05$) and *Bacteroides-Prevotella-Porphyromonas* ($P < 0.05$) in jejunal digesta were lower in pigs fed the high-CaP diets, irrespective of the protein source. Additionally, the gene copy numbers of *Enterobacteriaceae* in the jejunum and the caecum were significantly ($P < 0.05$) higher for the pea meal-based diets compared to the soybean meal-based diets, but the ammonia concentration was significantly lower ($P < 0.01$) in the caecum of pigs fed the pea meal-based diets, irrespective of their P content. There was a tendency ($P < 0.1$) for lower proliferation to ConA in pigs fed the high-CaP diets both in the ileal and jejunal mesenteric lymph node lymphocytes. No dietary effect could be determined for the lymphocyte proliferation to PWM of the jejunal and ileal mesenteric lymph nodes.

Conclusion: Results showed that dietary CaP might affect the lymphocyte proliferation to ConA. In addition, diets high in CaP reduced abundance of mainly proteolytic bacterial groups, thereby possibly lowering the risk for intestinal disturbances. Furthermore, protein source had an influence on mainly proteolytic bacteria assessed as well as on bacterial activity measured as ammonia concentration. Although abundance of mainly proteolytic *Enterobacteriaceae* in the caecum was higher for pea meal-based diets when compared to soybean meal-based diets, the observed lower ammonia concentration may indicate reduced proteolytic fermentation activity, which may be favorable for gut health.

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140. Impact of the dietary phosphorus level on growth performance and specific metabolic processes in a modern genetic line - a pilot study in piglets

Effekte des Phosphorgehaltes in Futtermitteln für Ferkel auf Wachstumsparameter, Fermentationsprodukte des intestinalen Mikrobioms, Knochenstabilität und –mineralisation sowie molekulare Merkmale und Stoffwechselwege – eine Pilotstudie

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Phosphorus (P) is an essential element in pig nutrition, which influences growth performance, the intestinal microbiome, blood buffering, bone mineralisation and various cell functions and molecular processes. Excess dietary P-levels have unfavourable effects on the environment and lessen natural P-resources. It has to be investigated whether the genetic setting of modern pig strains allows a reduced dietary P-supply in accordance with animals` requirement. Therefore, the aim of this pilot study was to investigate the impact of various P-levels on growth performance, bones' stability and mineralisation as well as molecular features and metabolic routes responsible for the dietary P-utilisation.

Methods: 18 weaned 28d old, castrated male and female piglets were divided into 3 groups. The piglets with an initial average body weight of 8.7 ± 0.88 kg were individually caged on a flat deck and were fed a pelleted diet *ad libitum* based on wheat, barley, soybean meal, and whey powder for 5 weeks. The calculated digestible P content was set at 0.29% (P-low), 0.48% (P-medium), and 0.66% (P-high), respectively, and the calcium content at 1.05% in each group. Body weight gain (BWG), feed intake and feed conversion ratio (FCR) were determined weekly and short chain fatty acids, lactate and NH_3 were analysed in faeces after week 1, 3, and 5. Peripheral blood mononuclear cells were collected at day 26, 35, 49, and 63 of life and differential gene expression was assessed using the Affymetrix microarray platform. Relevant hormone levels (osteocalcin, parathyroid hormone, 25-hydroxycholecalciferol) were analysed and radiologic studies on bone characteristics were performed after slaughtering the animals at the end of the trial. Significant differences were considered at $P<0.05$.

Results: The analysed P content corresponded to the calculated values, whereas the analysed calcium content in group P-low and P-medium amounted 0.9% and was slightly lower than calculated. Irrespective of the P level, neither differences in body weight gain nor feed intake were observed. However, the feed conversion was temporarily elevated and the faecal acetic acid content was significantly reduced ($p < 0.05$) in P-low in the second week. The dietary differences in digestible P-content were reflected by the levels of serum phosphate and parathyroid hormone (lowered due to P-low) and calcium or vitamin D (increased due to P-low) as well. The P-supplementation above requirements persistently affected animals as shown by microstructural parameters, including increased trabecular bone mineral density, trabecular number and Structure Model Index. Furthermore, the reorganization of osseous tissue was reflected by altered abundances of transcripts associated with bone morphology as shown by microarray analyses.

Conclusion: The study revealed considerable organismal plasticity of the animals in response to modulated P-supplementation and functional biodiversity regarding coping with dietary challenges. Genes found to be differentially expressed due to variable P-supply are involved in pathways relevant to P-utilisation and are potential candidate genes for improved P-efficiency.

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141. Influence of high levels of dietary zinc oxide on expression of genes involved in zinc and copper metabolism in the kidney of weaned piglets

Einfluss eine hohen Gehaltes an Zinkoxid im Futter auf die Expression von Genen des Zink- und Kupferstoffwechsels in der Niere von Absetzferkeln

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Question: Interaction of metal ions such as zinc and copper is well established for decades (1). However, the interaction may not only occur at the intestinal level but also post absorptive due to antagonistic effects at binding sites. During several independent experiments, we have analysed the trace element concentration (Zn, Cu, Mn, Fe) in various tissues of piglets fed normal (100 to 150 mg/kg, according to dietary recommendations) or very high (2000 to 3000 mg/kg) dietary zinc levels with no change in dietary concentration of other trace elements. Correlation analysis of trace elements in different tissues revealed a strong positive relationship (R^2 = 0.7 to 0.8) between zinc and copper accumulation in the kidney. Thus, we were interested whether the concomitant copper accumulation in the kidney was associated with genes relevant for copper homeostasis.

Methods: A total of 40 weaned piglets were randomly allocated into two treatment groups with 16 and 24 piglets each. The 16 piglets received a diet containing normal zinc concentration (NZn; 100 mg Zn/kg), whereas the other 24 pigs received very high dietary zinc (HZn; 2500 mg Zn/kg). After two weeks, 8 piglets from each treatment were killed and organ samples were taken. Eight piglets from the remaining 16 pigs fed HZn diets were subsequently subjected to NZn diets. All remaining piglets (n = 24) were killed after another two experimental weeks for organ sampling. Kidney mRNA expression of zinc transporter ZnT1, genes involved in copper metabolism (Ctr-1, ATOX-1, SOD-1, ATP7A, CCS, CP) and divalent metal ion transport (DMT-1) and binding (MT-1a, MT-2b, MT-3) was determined. Copper and zinc accumulation was further demonstrated in kidney cryo sections by rodanin and dithizon staining, respectively. Means were compared by ANOVA and Tukey HSD test using SPSS (version 21.0, Chicago, USA).

Results: No clinical signs of zinc toxicity or copper and iron deficiency were observed. Zinc and copper correlation was R^2 = 0.72 ($P < 0.05$) in kidney. Zinc and copper concentration in kidney tissue of pigs subjected to NZn diets for two weeks after two weeks of HZn feeding was similar to those fed NZn diets for the entire experimental period. Gene expression analysis revealed no significant differences for copper chaperones, copper transporters and copper-dependent factors. Expression of MT-1a, MT-2b and MT-3 were significantly higher in HZn fed pigs with most pronounced effects for MT-1a > MT-2b > MT-3. However, gene expression of MT-1a, MT-2b and MT-3 in pigs fed NZn diets after HZn feeding did not differ from pigs fed NZn diets only. Expression of ZnT1 was only higher in pigs fed HZn diets for four weeks. Histological demonstration of zinc and copper revealed co-accumulation in areas of renal medulla, which is consistent with previous reports in rodent models of heavy metal accumulation in areas of proximal tubuli (2).

Conclusions: The data suggest that high dietary zinc feeding in pigs leads to secondary copper accumulation in the kidney of pigs without influencing expression of genes relevant to copper metabolism. The concomitant copper accumulation appears to be a result of zinc dependent induction of MTs, which have a higher binding affinity to copper over zinc (3). However, the accumulation of copper and zinc is reversible within 2 weeks after withdrawal of HZn diets.

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3) Banci et al (2010): Nature, 465; 645-648.

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142. Secondary alimentary hyperparathyroidism in meerkat (*suricata suricatta*)

Knochenstoffwechselstörung durch Ca-Mangel bei einem Erdmännchen (suricata suricatta)

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A 14-week-old male pet meerkat (Suricata suricatta), weighing 0.48 kg, was referred to the small animal clinic "Laska" in Moscow with a fracture of the distal left femur. The injury happened the previous day when the meerkat fell to the floor. The basic diet was meat. X-Rays showed low radiographic density of bones. The bone was set surgically by a Kirschner pin. Brittleness of bones and thin cortical tissue made setting difficult. Nutrition history: Main diet compound was chicken breast (raw or cooked), some insects (Zophobas morio, Gromphadorhina portentosa, not fed or dusted with a high calcium diet), quail eggs, cheese; paprika, tomatoes, cucumber, marrow, cabbage, lettuce, apples, pears, corn, bananas; 150 mg of the mineral supplement "8 in 1 Excel Calcium for puppies", and 160 mg of the supplement "8 in 1 Multivitamin". Estimated Ca intake was 271 mg Ca/kg BW/day, phosphorus intake 410 mg/kg BW/day, Ca/P ratio was 0.7. The AZA (2011) uses analogias to cats, and mink for recommendations. NRC (2006) recommends approximately 500 mg Ca/kg BW and 450 mg P/kg BW for a growing kitten. The animal refused to eat calcium dusted insects or calcium carbonate. For hygienic reasons (small children in the house) the owner did not want to feed whole vertebral prey such as one day chickens or mice. Therefore chicken parts with soft bones (3rd phalanx of chicken wings), more insects, less vegetables and supplementation with calcium citrate and vitamins with taurine for cats, resulting in a total intake of 583 mg Ca/kg BW/day, 417 mg P/kg BW/day, and a Ca/P ratio of 1.4 were recommended. Dietary changes were carried out shortly after surgery. Four weeks after surgery the animal broke cage rest and damaged the cerclage. In renewed surgery the bones were less brittle than before. The femoral bone recovered during the following 3 months. Bone recovery was controlled with X-Rays monthly and intraoperative twice, when damaged cerclage was removed (four weeks after injury) and Kirschner pin was removed (four months after injury). Bone density was controlled with X-Rays, bone brittleness and cortical tissue was examined during the surgery. Full reparation (physiological appearance of bone densitiy in X-rays and macro-anatomic appearance as well as functional stability of bones during surgery for pin and cerclage removal, as well as during livelier movements of the animal) was only after 4 months, which is an unusually long time for a meerkat to recover. Meerkats without metabolic bone disease show fracture healing within three to four weeks. The present case suggests that Meerkats are prone to the typical metabolic bone disease observed in various carnivorous species eating a low calcium diet with an inverse Ca/P ratio.

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143. Determination of the upper critical concentration of sulfur containing amino acids in diets of juvenile turbot (*Psetta maxima*)

Bestimmung des oberen Grenzwertes für schwefelhaltige Aminosäuren in Rationen des juvenilen Steinbutts (Psetta maxima)

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The high need of crude protein (CP >50% in diet) (1) of carnivorous finfish species like turbot (*Psetta maxima*), is usually met by high inclusion levels of fish meal. Great efforts are being done to replace fish meal due to its low sustainability. However, high ratios of replacement proteins can lead to an imbalanced supply with sulfur containing amino acids (Methionine, Met; Cyst(e)ine, Cys). Beside deficiencies, oversupplies as well with Met+Cys could impair fish performance (2). If diets mainly contain ingredients rich in Met+Cys (e.g.: canola, wheat gluten, feather meal), the concentration could increase up to 3% in diet dry matter (DM). Therefore, it was the target of this study to evaluate, whether a maximum critical concentration for Met+Cys could be identified for practical diets of juvenile turbot.

Methods: In a dose-response trial, five different isonitrogenous and isocaloric diets (590 g CP; 22.35 MJ GE/kg DM) were fed to respective three tanks (150L) in a recirculating aquaculture system. Every tank was stocked with 20 juvenile turbots (initial mean weight 30.9 g s.d. 3.2). Diets only differed in Met+Cys concentration from 1.8 to 3.0% Met+Cys in diet DM. This was realized by increments of 0.2% L-Met and 0.1% L-Cys per step, to ensure a similar Met:Cys ratio (0.62-0.64) for all diets. The fishes were fed until apparent satiation once a day. The experiment lasted 56 days, and daily feed intake and growth (% of body weight (relative feed intake, RFI; relative growth, RG)) were determined. To identify the upper critical concentrations of Met+Cys a segmental linear regression analysis (plateau-decrease) was applied using R software 3.1.1.

Results: Mean values of RG and RFI are given in the table, showing a plateau and a decrease at the highest Met+Cys concentrations (oversupply). According to the statistical model the upper critical concentration for RFI is 2.6% (± 0.5 s.e.) Met+Cys in diet DM (4.4% in CP) and for RG 2.3% (± 0.4 s.e.) in diet DM (3.9% in CP), respectively. Both breakpoints are significant (P<0.001).

Table: Impact of a moderate oversupply by Met+Cys on relative growth (RG) and relative feed intake (RFI) per day on juvenile turbot (mean ± s.d.; n=3).

Met+Cys (% in DM)	1.8	2.1	2.4	2.7	3.0
RG (%)*	1.78 ± 0.03	1.81 ± 0.06	1.77 ± 0.02	1.68 ± 0.17	1.63 ± 0.15
RFI (%)**	1.19 ± 0.02	1.21 ± 0.03	1.21 ± 0.00	1.15 ± 0.11	1.16 ± 0.07

*RG (relative growth) = [ln(final live weight) - ln(initial live weight)] * 100 / feeding days

**RFI (relative feed intake (DM) in % of daily feed intake per live weight)

Conclusions: For RFI and RG the upper critical concentrations are lying in ranges which could be achieved in practical diets for turbots containing high amounts of protein sources rich in Met+Cys. These upper levels could be defined less distinctly, indicated by the relatively high s.e., than the lower in previous studies (2).

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144. Influence of graded dietary L-arginine (Arg) supply on growth and N balance in female chicks of four purebred layer genotypes (GT) differing in phylogeny and performance

Einfluss diätetischen L-Arginins auf Leistung und Stickstoffbilanz weiblicher Küken vierer Genotypen von Reinzuchtlegehennen im frühen Lebensalter

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As avian urea cycle is functionally incomplete, Arg is regarded to be essential in birds. It plays a decisive role in metabolic pathways and acts as precursor of nitric oxide which serves multiple functions in vasodilatation, immunology and neurotransmission. Due to genetically determined differences in phylogeny and performance, it can be hypothesized that Arg requirement and utilization differ between studied GT. Therefore, effects of graded dietary Arg supply on growth and N balance were examined in female chicks of four GT differing in phylogeny and performance (WLA/R11: high/low performing white; BLA/L68: high/low performing brown).

Methods: A total of 72 female one day-old chicks were housed in pairs in metabolism cages with feed and water *ad libitum* for the first four weeks. Each GT was evenly distributed to three diets containing 0.68%, 1.00% and 2.00% Arg (n=6). In week five a N balance trial of 7 days was carried out with restricted feed to 50 g/chick/d. Feed not consumed was weighed back. All excrements were collected twice a day, stored at -20°C and freeze-dried. Excrements and diets were analysed for dry matter and Kjeldahl N according to the methods of VDLUFA. The ANOVA was carried out with a two factorial design (GT and ARG) by using Tukey-Kramer test within procedure MIXED (SAS 9.2). Differences between fixed factors were considered as statistically significant for $p<0.05$.

Results: GT and Arg influenced performance in the first four weeks ($p<0.05$; Table). L68 achieved highest daily weight gain and feed intake ($p<0.05$). The obtained performance was lower by feeding 0.68% Arg than by feeding higher grades ($p<0.05$). 2% Arg caused a higher N intake and retention ($p<0.05$) in GT, in which L68 had highest N intake and retention ($p<0.05$). In contrast to productive protein value (PPV), protein efficiency ratio (PER) was significantly affected by GT. Brown GT as well as high performing GT utilized N more efficient for weight gain than their counterparts ($p<0.05$).

		week 1 - 4				week 5				
GT	Arg (%)	DWG (g/d)	DFI (g/d)	FCR (g/g)		DWG (g/d)	N intake ($g/kg^{0.67}/d$)	N retention ($g/kg^{0.67}/d$)	PER	PPV (%)
WLA	0.68	6.3	18.6	3.15		11.9	2.42	1.38	2.16	57.1
	1.00	7.5	19.6	2.84		12.8	2.41	1.41	2.15	58.4
	2.00	7.2	19.9	3.04		15.5	2.70	1.52	2.35	56.5
BLA	0.68	5.9	17.0	3.21		12.9	2.25	1.32	2.57	58.8
	1.00	7.0	17.6	3.08		15.6	2.43	1.42	2.70	58.3
	2.00	6.2	17.7	4.26		17.2	2.80	1.62	2.64	58.0
R11	0.68	5.8	19.6	3.59		10.6	2.49	1.51	1.98	60.1
	1.00	6.0	17.3	3.23		9.0	2.40	1.50	1.70	62.6
	2.00	6.1	19.4	3.54		12.3	2.90	1.66	1.89	57.4
L68	0.68	7.4	19.8	2.77		15.7	2.72	1.57	2.30	57.8
	1.00	9.1	23.5	2.67		20.3	2.83	1.75	2.51	61.7
	2.00	7.9	22.5	3.18		18.4	3.21	1.91	2.28	59.4
SEM		0.3	0.5	0.22		1.0	0.06	0.06	0.11	1.7
ANOVA (p value)										
GT		< 0.001	< 0.001	0.001		< 0.001	< 0.001	< 0.001	< 0.001	0.1863
ARG		< 0.001	0.0127	0.0035		0.0014	< 0.001	< 0.001	0.8796	0.1417
GT x ARG		0.3775	< 0.001	0.1314		0.0927	0.1816	0.5424	0.2393	0.6951

Conclusions: In early life stage Arg supply has an appetizing and growth enhancing effect. Latter one is characterised more closely by differences between PER and PPV. As retention of ingested N (PPV) is neither GT nor Arg specific, the GT utilize N for weight gain with different efficiency (PER). Therefore, it can be assumed that the weight gain of GT is not only characterised by pure N retention in form of muscle growth during early life stage and body composition shal be examined.

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145. Effects of glycine supplementation of low protein diets with low or high proportion of free amino acids on growth and protein utilisation in broilers

Auswirkungen eines Glycinzusatzes zu Futtermischungen mit geringem Rohproteingehalt bei geringem und hohem Anteil freier Aminosäuren auf Wachstum und Proteinverwertung bei Masthühnern

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Lowering the crude protein (CP) concentration of broiler diets is effective in decreasing meat production-related N emission. A reduction of CP concentration along with adequate amino acid (AA) concentrations often leads to an increased proportion of free AA in the diet. Because free AA are assumed to be absorbed faster than protein-bound AA, an imbalance in the systemic AA circulation might lead to increased AA catabolism. Ammonia detoxification to uric acid requires glycine. This study investigated the responses of broilers to glycine supplements at different dietary levels of free AA.

Methods: Birds were fed a commercial starter diet until d 7 post hatching and reaching average body weight 161 g. Then they were distributed to 48 floor pens of 10 birds each (Exp. 1) and 48 metabolism cages of 2 birds each (Exp. 2). From d 7 to 21, 12 pens or cages were allocated to four experimental diets in a 2×2 factorial arrangement. A semi-purified basal mixture consisting mainly of corn, casein and corn starch was used. The diets contained 170 to 190 g CP per kg DM and the ratio of essential AA was adjusted to GfE recommendations. Diets contained either soy protein isolate (SPI) at a level of 79 g/kg or a mix of free AA that supplied the same amount of all AA. Intended AA concentrations were confirmed by diet analysis. As serine is equally effective in meeting the functions of glycine on a molar basis (1) the glycine equivalent (G) of both AA was calculated. A mix of glycine and l-serine was used to achieve low and high (12.0 and 20.5 g/kg DM) levels of dietary G. Average daily gain (ADG), average daily feed intake (ADFI) and gain:feed ratio from 7 to 21 d were the response traits. In Exp. 2 excreta were collected quantitatively at d 21 in 8 h intervals for determination of N accretion. Statistical evaluation was done using the MIXED procedure of SAS.

Table: Response of broilers to different AA sources and concentrations of glycine equivalent (G)

	Response trait	SPI		AA-Mix		SEM	ANOVA (P-values)		
Glycine		low	high	low	high		S	G	G×S
Exp. 1	ADFI (g/d)	55.0^{b}	58.6^{a}	37.7^{c}	36.4^{c}	0.76	***	0.13	**
	ADG (g/d)	42.9	46.7	22.9	24.2	0.72	***	**	0.11
	Gain:feed (g/g)	0.78^{a}	0.80^{a}	0.61^{c}	0.66^{b}	0.01	***	**	*
Exp. 2	ADFI (g/d)	54.2^{b}	59.0^{a}	39.9^{c}	36.0^{c}	1.28	***	0.72	**
	ADG (g/d)	42.3^{b}	47.1^{a}	23.0^{c}	22.6^{c}	1.09	***	*	*
	Gain:feed (g/g)	0.78	0.80	0.58	0.63	0.01	***	*	0.31
	N accretion (g)	1.74^{b}	2.13^{a}	1.08^{c}	0.98^{c}	0.09	***	0.12	**
	N efficiency (g/g)	0.75	0.76	0.73	0.73	0.01	0.09	0.79	0.70

Results: ADG, ADFI and gain:feed were similar in both experiments and below breeder's objectives. Replacement of SPI with the AA mix significantly decreased ADFI and gain:feed and hence ADG. The high G level raised ADG. The G level had no effect on gain:feed in diets with SPI but increased gain:feed in diets with the AA mix. N accretion resembled ADG but N efficiency was unaffected by treatment.

Conclusions: Effects of G supplementation depend on the dietary proportion of free AA. Even when the AA supply is not changed, replacing a substantial amount of SPI with free AA in low protein diets impairs growth, feed efficiency and N accretion mainly due to reduced feed intake. The efficiency of N accretion did not depend on the dietary proportion of free AA or the G level.

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146. **Effect of dietary methionine to cystine ratio on methionine efficiency in growing meat type chicken**

Einfluss des Methionin:Cystin-Verhältnisses im Futter auf die Methioninwirksamkeit bei Masthähnchen

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In studies on methionine (Met) efficiency or Met requirement, both the dietary concentration of total sulfur containing amino acids (TSAA) and cystine (Cys) are in focus according to their considerable importance also for feather formation. If the dietary Cys supply is not adequate, Met will be degraded to form Cys and is not available for other metabolic functions. Therefore, the objective of the current study was to add to present knowledge about optimal dietary Met to Cys ratio for meat type chicken and its effect on observed Met efficiency data for further modeling of Met requirements.

Methods: Two experiments were conducted with a total of 282 male meat type broiler chickens (ROSS 308): N balance study (Exp.1) with two individual consecutive 5d collection periods both during starter (d10-20, N=36) and grower period (d25-35, N=36); growth study (Exp.2; d1-36, N=210) with whole body N analyses for assessing N deposition (1). The control diet I based on corn, field peas, soybean meal and soybean protein concentrate was limiting in Met supply (0.27% Met, 19.5% CP in the starter; 0.25% Met, 18.0% CP in the grower period). Graded supplementation with 0.075% Met (II), 0.15% Met (III) and 0.15% Met+0.16% Cys (IV) yielded the experimental diets II, III, IV. Data analysis utilized principles of an exponential N utilization model (2, 3) for evaluating both dietary protein quality *(b)* and Met efficiency *(bc^{-1})* as relative data (diet I=100) in the table. One-way ANOVA (SPSS software package) connected with the Tukey-test run to identify significant differences between variables (p

Results: As expected, protein quality parameter *(b)* responded significantly due to the Met concentration (c_{Met}) between diets I-III. Additional Cys (diet IV *vs.* III) generally yielded enhanced protein quality *(b)* and Met efficiency *(bc^{-1})* indicating that Met:Cys ratio in diet III was suboptimal. In diet III as compared to diet II, the Met efficiency *(bc^{-1})* was significantly (starter, Exp.1) or numerically lower. This effect was partly compensated by additional Cys (L-Cys·HCl·H_20; diet IV), supporting the assumed suboptimal Cys supply with diet III.

Diet	I	II	III	IV
c_{Met} (g/16gN)	1.40	1.80	2.19	2.17
Met/Cys supplementation (%)	-	0.075/-	0.15/-	0.15/0.16
Met:Cys (% of TSAA)	48:52	54:46	59:41	48:52
Relative protein quality (b)				
Exp. 1: N balance (Starter)	100[a]	123[b]	134[c]	147[d]
N balance (Grower)	100[a]	137[b]	161[c]	174[c]
Exp. 2: Growth study	100[a]	133[b]	157[c]	171[d]
Relative Met efficiency (bc^{-1})				
Exp. 1: N balance (Starter)	100[a]	98[a]	88[b]	96[a]
N balance (Grower)	100[a]	107[a]	102[a]	111[a]
Exp. 2: Growth study	100[a]	104[ab]	101[a]	110[b]

Conclusions: Supplementation of Met or Met and Cys to a Met limiting diet significantly improved the dietary protein quality. These results are in line with zoo-technical data and protein deposition data observed in a corresponding growth trial (1). However, the obtained dietary Met efficiency data were influenced by the dietary Met:Cys ratio and responded depending on the age period of the chicken. During starter period, the decline of observed Met efficiency indicates the need for a higher proportion of Cys in the TSAA for feather formation. In consequence, this factor needs definitely more attention when requirement studies are designed both with traditional supplementation technique and diet dilution technique.

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147. Influence of grinding intensity on the precaecal amino acid digestibility from legumes in laying hens

Einfluss des Vermahlungsgrades auf die precaecale Aminosäureverdaulichkeit aus Leguminosen bei Legehennen

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In broilers it already was examined that particle size differently affects amino acid digestibility for various feed ingredients (1). Whether there is a similar effect of grinding intensity on the precaecal digestibility (pcD) of crude protein (CP) and amino acids (AA) in laying hens should be investigated.

Methods: As test ingredient LEGUMI-therm® (LGT) a thermically treated and partially dehulled mixture of ⅓ treated faba beans, field peas and sweet lupins each originated from organic farming was selected (CP: 265, crude fat: 22, crude fibre 72 g/kg). LGT was ground (hammer mill) using different sieve sizes: 2.0 mm (S), 3.5 mm (M), 5.0 mm (L). For the trial 7 pelleted diets were created. In the basal diet (BD) maize, wheat gluten, maize starch as main components and TiO_2 (5 g/kg) as indigestible marker were included. In the remaining diets 100 or 200 g/kg LGT were added instead of maize starch. 210 pullets (Lohmann Brown) were randomly assigned to 7 dietary treatments, 6 replications with 5 hens each. For the collection of digesta hens were killed by asphyxiation of CO_2 after an *ad libitum* feeding period of 5 days (24th wk). The section between Meckel`s diverticulum and 2 cm anterior the ileo-caeco-colonic junction was eviscerated (2). The content was flushed out with distilled water and pooled within one replication (5 hens). Total N, AA and TiO_2 were analysed in diets and freeze-dried ileal digesta. PcD of AA and CP was calculated by a linear regression approach (3). Differences in pcD between the different sieve sizes were tested at a level of significance of $P < 0.05$.

Results: Average daily feed intake ranged between 111 and 144 g. Sieve analysis of LGT showed distinctly different percentages of mass >1mm: 1% (S), 20% (M), 38% (L). The pcD of AA of LGT (estimate ± SE, in %; see table) was not affected by varying grinding intensity.

	Sieve pore size							P		
	S		M		L			S to M	S to L	M to L
Crude Protein	88	± 5.3	81	± 5.1	85	± 5.3		0.388	0.665	0.669
Arginine	92	± 1.8	88	± 2.4	91	± 2.1		0.247	0.806	0.385
Isoleucine	89	± 3.1	82	± 3.9	85	± 4.4		0.176	0.488	0.606
Leucine	89	± 3.7	84	± 4.1	87	± 4.7		0.337	0.784	0.555
Lysine	90	± 2.6	85	± 3.6	87	± 4.1		0.251	0.516	0.713
Methionine	90	± 4.0	86	± 4.6	90	± 5.3		0.575	0.912	0.556
Phenylalanine	90	± 5.2	85	± 5.5	84	± 6.7		0.544	0.522	0.925
Threonine	81	± 5.4	76	± 5.9	79	± 6.3		0.528	0.805	0.725
Valine	87	± 3.5	80	± 4.4	82	± 5.1		0.219	0.414	0.773

Conclusion: This study indicates that generated different grinding intensity of LGT has no influence on the pcD of AA and CP in laying hens. However, further investigations are necessary to verify the impact of grinding intensity for single feed. This is needed to recommend an 'optimal' particle size for standardized measurements of pcD of AA in laying hens.

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148. Relations between the crude protein content and the amino acid profile of organically produced field beans (*Vicia faba* L.) and field peas (*Pisum sativum* L.)

Effekte hoher Rohproteingehalte auf das Aminosäurenmuster ökologisch angebauter Ackerbohnen (Vicia faba L.) und Futtererbsen (Pisum sativum L.)

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In Organic Farming, grain legumes are important protein feedstuffs. There are hints indicating that the amino acid (AA) profile is affected by factors associated with varying crude protein (CP) contents of crops [1]. The knowledge on this relationship between CP and AA profile needs to be extended to optimize feeding strategies for monogastrics as well as the selection of cultivars and varieties in fodder crop cultivation.

Methods: Samples of field beans (*Vicia faba* L.) and field peas (*Pisum sativum* L.) were collected from field trials of various organically managed experimental locations in Germany in the years 2011, 2012 and 2013. Altogether 86 field pea and 67 field bean samples were dried and ground to pass through a 0.5 mm sieve for AA analyses and a 1 mm sieve for CP analyses. Subsequently, the contents of CP (according to the VDLUFA method (Kjeldahl, N*6.25)) and AAs (with HPLC) were analyzed. Pearson correlation analyses between the content of CP (g $100g^{-1}$ DM) and the amounts of AAs (g $100g^{-1}$ CP) were conducted with R. To find differences in the CP and AA contents between the cultivars, Kruskal-Wallis-Tests and the multiple comparison procedure kruskalmc [2] were used.

Results: Compared to tabular values derived from conventional samples [3] organically produced field beans contained similar amounts of CP, whereas the CP content of field peas was lower. The protein of field peas contained significantly ($p < 0.05$) larger amounts of the essential and semi-essential AAs except threonine, histidine and arginine. Thus, the relations between CP and AA differed between field beans and field peas. In field beans, the limiting AAs (for swine and poultry) lysine, methionine, and cysteine were negatively correlated with the CP content. However, the field pea sample revealed negative correlations between the amount of CP and the contents of methionine, threonine, isoleucine, and valine. The content of arginine increased with decreasing amounts of CP in both grain legume cultivars. The correlation coefficients as well as the mean contents of CP (g $100g^{-1}$ DM) and the essential and semi-essential AAs (g $100g^{-1}$ CP) are shown in the table.

Cultivar	N		CP	Lys	Met	Cys	Thr	His	Ileu	Leu	Val	Phe	Tyr	Arg
Vicia faba	67	M	29.78	6.29	0.72	1.10	3.39	3.26	3.80	7.02	4.26	4.15	4.42	8.69
		SD	1.53	0.40	0.09	0.14	0.25	0.42	0.17	0.34	0.18	0.35	0.36	0.53
		R		-0.41*	-0.41*	-0.47*	-0.16	-0.15	-0.22	-0.18	-0.20	-0.23	-0.12	0.49*
Pisum sativum	86	M	21.21	8.17	1.03	1.40	3.96	3.17	4.21	7.23	4.73	4.99	4.73	7.65
		SD	2.14	0.76	0.09	0.20	0.17	0.56	0.21	0.33	0.21	0.30	0.27	0.57
		R		-0.24	-0.54*	-0.19	-0.55*	0.11	-0.41*	-0.24	-0.44*	-0.06	-0.15	0.41*

N = number of samples, M = mean, SD = standard deviation, R=correlation coefficient, *=p

Furthermore, the contents of proline (R = -0.42) and glycine (R = -0.35) were negatively correlated with the CP content of field peas ($p < 0.01$). No notable correlations between the non-essential AAs and CP were found in field beans.

Conclusions: The results demonstrate that high CP contents in grain legumes are not the only factor to be considered, when protein feedstuffs for monogastrics in Organic Farming are needed. Although significant negative correlations between essential AA and CP contents are repeatedly found, it is not observed that non-essential AAs mainly account for these changes. Nevertheless, a decline of the amounts of lysine and sulfur-containing AAs negatively affects the quality of crude protein. Further studies with large samples are needed to detect the influence of environmental and genetic factors.

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[2] GIRAUDOUX, P. (2013): pgirmess: Data analysis in ecology. R package version 1.5.8.

[3] DLG (2014): DLG-Futterwertabellen-Schweine. DLG-Verlag GmbH.

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149. Standardized ileal amino acid digestibility in eight genotypes of triticale fed to growing pigs

Standardisierte praecaecale Verdaulichkeit von Aminosäuren in acht verschiedenen Triticale-Genotypen für Schweine

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Due to their high dietary inclusion level, cereal grains can supply up to 60% of the pigs' total amino acid (AA) and crude protein (CP) requirement. In the last 3 decades, numerous studies have been conducted to determine the nutritional value of cereal grains, such as wheat and barley, but until now there are only few reports on standardized ileal digestibility (SID) of AA in triticale for pigs. Therefore, the aim of the present study was to determine the variation in chemical composition and SID of CP and AA in 8 triticale genotypes, which have recently been added to the German Descriptive Variety List.

Methods: A total of 8 barrows with an average initial body weight (BW) of 31±2 kg were fitted with simple T-cannulas at the distal ileum. The animals were allotted to an 8×8 Latin square design with 8 periods of 7d each and 8 pigs receiving a total of 8 assay diets. An N-free diet was fed in an additional period at the conclusion of the experiment to determine basal ileal endogenous CP and AA losses. In total, 8 winter triticale genotypes (T1 to T8) were evaluated. Triticale was the sole source of CP and AA in the diet. Titanium dioxide was used as an indigestible marker. During every period, pigs were fed the experimental diets at a daily level of 4% (as fed) of their average BW. Ileal digesta samples were collected continuously for a total of 24 h. Data were analyzed using the ProcMixed of SAS.

Item	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P-value
Content of CP and AA (g/kg, as-fed)										
CP	106	107	109	109	114	117	120	121	-	-
Lys	3.3	3.2	3.5	3.5	3.5	3.8	3.6	3.6	-	-
Met	1.7	1.7	1.7	1.7	1.7	1.9	1.8	1.8	-	-
Thr	3.2	3.2	3.3	3.3	3.4	3.5	3.5	3.6	-	-
Trp	1.2	1.2	1.1	1.2	1.2	1.3	1.1	1.2	-	-
SID of CP and AA (%, LSMeans)										
CP	81	83	83	84	85	83	83	84	1	0.26
Lys	72	73	73	75	77	74	75	73	2	0.48
Met	84	85	84	86	87	85	85	85	1	0.25
Thr	73	75	75	77	77	76	74	75	2	0.58
Trp	80	81	79	83	82	81	80	80	2	0.60

Results: Among the 8 triticale genotypes, the CP content varied between 106 and 121 g/kg in T1 and T8, respectively. The SID of CP, ranging from 81% in T1 to 85% in T5, did not differ between the triticale genotypes but standardized ileal digestible content (cSID) of CP ranged from 8.4 in T1 to 9.8% as-fed in T9 ($P<0.05$). Similarly, for most AA including Lys, Met, Thr and Trp, SID of AA in the 8 triticale genotypes did not differ, but cSID (%, as-fed) of Lys, Met, Thr, Trp ranged from 0.23 in T2 to 0.28 in T6, from 0.14 in T1, T2, and T3 to 0.15 in T6, from 0.23 in T1, T2, and T3 to 0.26 in all other genotypes, and from 0.07 in T3 to 0.09 in T6, respectively ($P<0.05$). Compared to SID values for triticale in current feed tables (1), the SID in the present 8 triticale genotypes were up to 2, 8, 2, and 2%-units lower for CP, Lys, Met, Thr, respectively, and up to 1%-unit higher for Trp.

Conclusions: Current triticale genotypes provide significant amounts of digestible AA to the pig with only small variation between individual genotypes. In the triticale genotypes used in the present study, SID of CP, Lys, Met, and Thr are lower compared to values in current feed tables. In particular, a lower content of SID of Lys in all 8 triticale genotypes should be taken into account in formulating pig diets.

1) NRC (2012): Nutrient requirements of swine, National Academy Press.

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150. Effect of different protein levels and sources in diets of weaned piglets on intestinal microbial composition

Einfluss unterschiedlicher Futterproteinquellen und -mengen auf die Zusammensetzung der intestinalen Mikrobiota abgesetzter Ferkel

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The composition of the intestinal microbiota is influenced by dietary factors. Especially during weaning, variations in dietary protein supply may beneficially affect microbial composition in the intestine to alleviate disorders, as undigested protein can be used as energy source for potential pathogenic bacteria. The present study was designed to examine the effects of casein-based vs. soybean meal (SBM)-based diets, each at 6 inclusion levels in the diet, on the composition of ileal and fecal microbiota of weaned piglets.

Methods: A total of 48 weaned piglets (initial BW of 6 ± 0.9 kg), fitted with simple T-cannulas at the distal ileum, was used in 4 consecutive experiments with 2 periods each. The piglets were fed semi-synthetic assay diets consisting of either graded levels of SBM (213, 338, 464, 589, 715, 840 g/kg as-fed basis) or casein (99, 157, 215, 274, 332, 390 g/kg as-fed basis) at the expense of cornstarch. Diets were supplemented with dextrose, lactose, cellulose, oil, and a mineral premix. The pigs received their assay diets at 2 feeding levels corresponding to 30 and 60 g/kg BW. Fresh feces and ileal digesta of each piglet were sampled to determine 16S rRNA gene copies of total eubacteria, *Lactobacillus* spp., *Bifidobacterium* spp., and *Enterobacteriaceae* by use of real-time PCR. The study was conducted as row-column design. Data were analyzed using the MIXED procedure of the Statistical Analysis System (SAS). All results are reported as least square means.

Results: Ileal counts of total eubacteria, and *Enterobacteriaceae* were higher ($P < 0.001$, data not shown) in the casein-based diets. Ileal counts of lactobacilli linearly increased ($P < 0.01$, data not shown), as the protein level was increased up to 335 g/kg. Fecal counts of all analyzed bacterial groups were higher ($P < 0.001$) for the SBM-based diets, except for *Enterobacteriaceae*, which were higher ($P < 0.05$) in the casein-based diet (Table). Fecal counts of total eubacteria and *Bifidobacterium* spp. linearly increased ($P < 0.01$, Table), as the protein level in the SBM-based diet was increased, while total eubacteria linearly decreased ($P < 0.01$) for increasing protein level in the casein-based diets.

Table

	Source	Level						Pooled	P-values		
		85	135	185	235	285	335	SEM	Level	Source	Level × Source
Feces											
Total eubacteria	SBM[a]	8.7	8.8	8.9	8.8	9.2	9.1	0.19	0.226	< 0.001	0.059
	CAS[b]	8.6	8.5	7.8	7.6	8.3	7.1				
Bifidobacterium spp.	SBM[a]	5.1	5.2	5.3	5.1	5.4	6.4	0.22	0.052	< 0.001	0.002
	CAS	5.6	5.2	5.0	4.7	4.9	4.8				
Lactobacillus spp.	SBM	6.4	6.9	7.2	6.8	7.3	6.9	0.29	0.788	< 0.001	0.697
	CAS	5.6	5.5	5.1	5.5	5.8	5.2				
Enterobacteriaceae	SBM	6.6	6.8	6.7	6.7	7.0	7.0	0.35	0.674	0.044	0.875
	CAS	7.2	7.0	7.0	6.9	7.4	6.8				

SBM, soybean meal; CAS, casein; data for feeding level not shown; level × feeding level, source × feeding level and level × source × feeding level not significant; [a] Positive linear effect of level. [b] Negative linear effect of level.

Conclusions: Both, protein level and protein source affect growth of bacterial groups differently. While high levels of SBM in combination with high fiber contents in the diet may promote beneficial bacteria, highly digestible protein sources may increase protein fermenting bacteria in piglet's intestine, thereby promoting intestinal disturbances. However, since diets did not only differ in protein digestibility, but also in carbohydrate content including fiber, the observed effects might be also attributed to other dietary factors in addition to protein source and level.

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151. Tissue distribution of branched-chain α-keto acid dehydrogenase activity in response to high leucine diets in weaned piglets

Aktivität der Verzweigtketten-alpha-Keto-Dehydrogenase in verschiedenen Geweben des Absetzferkels bei Gabe von leucinreichen Rationen

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Leucine (Leu) has - besides its role as a substrate for protein synthesis - vital function in stimulation of protein translation, which had motivated scientists to elucidate the efficacy of high-leucine diets to improve performance. Although it is known that high-leucine diets administered to pigs stimulate the activity of liver and muscle branched-chain α-keto acid dehydrogenase (BCKDH) which catalysis the degradation of branched-chain amino acids (BCAA), we have currently no data on BCKDH activity distribution in piglets except for liver and skeletal muscle.

Methods: To investigate the tissue distribution of porcine BCKDH activity in response to high-leucine diets, 120 crossbred [Pietrain x (Large White x Landrace)] barrows and female piglets which were blocked by sex, body weight and degree of relationship (half-siblings) and divided into three dietary treatment groups. Piglets received diets with SID Leu:Lys of 100% (control), of 200% and of 400%, respectively for 35 days. At the end of the study, from 10 piglets per group blood and tissue samples were collected. The amino acid (AA) concentrations in plasma were determined by HPLC (1). BCKDH activity was assayed spectrophotometrically (2).

Results: As expected the daily feed intake of piglets that received the high-leucine diets was lower than in the control group ($P<0.05$), but the gain:feed ratio was not affected by the treatments. Data show a dose-dependent increase of plasma leucine concentrations upon increasing SID Leu:Lys-ratios in the diet ($P<0.05$), while the plasma concentrations of valine and isoleucine decreased ($P<0.05$). Data reveal strong differences in BCKDH activity within the analyzed tissues (Table). The BCKDH activity was highest in pancreas followed by kidney, liver, cardiac muscle, brain, skeletal muscle and adipose tissue. SID Leu:Lys-ratios of 200% had only minor impact on BCKDH activity, whereas SID Leu:Lys-rations of 400% increased significantly the BCKDH activity in nearly all tissues, exept skeletal muscle and adipose tissue. The strongest stimulation of BCKDH activity upon excessive leucine was found in liver, cardiac muscle and brain.

Table 1 BCKDH activity of several tissues

	SID Leu:Lys				p-value
	100	200	400	SEM	
Pancreas	20.0ab	18.3a	26.7b	1.4	0.039
Kidney	11.8a	13.4ab	14.0b	0.3	0.008
Liver	10.8a	14.8ab	19.0b	1.2	0.012
Cardiac muscle	6.0a	5.8a	10.0b	0.4	0.000
Brain	2.1a	2.8a	5.2b	0.3	0.000
Skeletal muscle	0.7	0.9	0.8	0.1	0.275
Adipose tissue	0.4	0.7	0.7	0.1	0.043

Conclusion: This study shows that BCKDH activity in skeletal muscle is almost negligible, whereas the activity was highest in pancreas. The most marked stimulation of BCKDH activity upon high-leucine diets was found in liver, cardiac muscle and brain.

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152. **Effects of a varying crude protein supply on fattening performance of finishing Simmental bulls**

Einfluss unterschiedlicher Rohproteinzufuhr auf die Mastleistung von Fleckviehbullen in der Endmast

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The officially valid standards for crude protein (CP) supply to fattening bulls in Germany were published 1995 (GfE, 1995). Over the past years, however, the level of growth performance, as well as the final weight of fattening bulls has increased. This implies that CP requirements may have changed and need to be adjusted. For this reason, a dose-response study was conducted to evaluate CP requirement in fattening bulls in the finisher phase. The study was part of the Bavarian governmental project „Heimische Eiweißfuttermittel".

Methods: 66 Simmental bulls (BW: 490±31 kg; age: 320±9 d) were assigned equally to six feeding groups. All groups were fed *ad libitum* with a TMR based on maize silage, straw and concentrates, calculated to be comparable in energy concentration (11.5 MJ ME/kg TM) and in concentration of nutrients, except of CP. TMR of groups 1-5 had CP concentrations of 7.9, 9.8, 11.7, 13.6, and 15.5 % of DM, respectively. Group 6 was fed the TMR with 7.9 % CP supplemented with urea (0.73 % of DM) so that CP concentration of TMR was increased to 10.1 % CP of DM. Varying CP concentration was obtained by substitution of soy bean and rapeseed meal by dried beet pulp and wheat. Individual feed intake was automatically recorded daily while the live weight was recorded every four weeks. Blood was collected every three months. The bulls were slaughtered at an average age of 514 ± 3 days. The data were evaluated by a one-factorial ANOVA using SAS. CP requirement was calculated by broken-line model.

Results: Feed and ME intake were highly affected by CP supply with lowest values in group 1 and highest in group 4. CP intake increased ($p<0.05$) with increasing CP concentration of TMR. End weight and daily gains increased up to a dietary CP concentration of 13.6 % of DM (group 4). Higher CP concentration of 15.5% of DM in group 5 led to slightly lower end weight and gain. Addition of urea failed to improve feed intake or performance over the level in group 1 despite the elevated CP intake. Dietary CP requirement as calculated by broken-line model was 12.2 % of DM, corresponding to a daily CP intake of 1310 g or a CP/ME-ratio of 10.9 g/MJ, respectively.

Group	1	2	3	4	5	6 (urea)
CP, % of DM	7.9	9.8	11.7	13.6	15.5	10.1
DM-intake, kg/d	9,46±0,85^{c}	10,04±0,77^{bc}	10,61±0,84^{ab}	11,22±0,67^{a}	10,53±0,68^{ab}	9,55±0,84^{c}
CP intake, g/d	752±71^{e}	989±76^{d}	1242±99^{c}	1520±91^{b}	1628±105^{a}	964±84^{d}
ME intake, MJ/d	105±9^{c}	112±9^{bc}	119±9^{ab}	126±8^{a}	119±8^{ab}	105±9^{c}
Final weight, kg	706±49^{b}	742±57^{ab}	780±42^{a}	792±50^{a}	777±43^{a}	710±52^{b}
Daily gain, g	1053±156^{b}	1231±290^{b}	1491±130^{a}	1584±203^{a}	1498±135^{a}	1083±174^{b}
Feed/gain, kg/kg	9,08±0,93^{a}	8,73±2,94^{a}	7,14±0,52^{b}	7,15±0,64^{b}	7,05±0,41^{b}	9,01±1,58^{a}

Conclusions: Results of the present study demonstrate that not only a marginal CP supply but also a slight oversupply tends to negatively affect performance of fattening bulls, even in the finishing phase. Optimum CP intake or CP concentration of diets appears high for finishing bulls what may be attributed to the high growth rates observed in our study. The optimum CP/ME ratio of 10.9 g/MJ is near the current recommendations. The lack of response due to urea addition is hard to explain and needs further investigation.

Table: Feed intake, CP and ME intake and growth performance (means ± SD)

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153. The occurence of gamma-amino-butyric-acid (GABA) in grass silages with different degree in proteolysis

Zum Vorkommen von Gamma-Amino-Buttersäure (GABA) in Grassilagen mit unterschiedlichem Proteolysegrad

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Question: The chemical nature of protein in grass silages shows considerable variations. An unavoidable proteolysis occurs in the freshly cut grass and additionally during the ensiling process independent from the botanical origin of protein. The present data refer to the loss in true protein in grass silages and the degree in GABA-formation.

Methods: Feeding data and samples of grass silages were collected on 16 dairy farms in northern Germany on the occasion of veterinary herd control. The feed samples were directed to proximate analysis including the analysis of true protein (TP) and ammonia-nitrogen (VDLUFA 2007/2008). Analysis of amino acids (AA) and GABA were processed in a subset of samples (Commission Regulation (EC) No 152 / 2009 of 27 January 2009, laying down the methods of sampling and analysis for the official control of feed, Fa. Evonik-Degussa, Hanau).

Results: The pH in silages averaged at 5.3 (Tab. 1), The dry matter (DM) varied between 231 and 789 g/kg. TP contributed on average 49 % to total N. GABA averaged at 2.9 g/16 N with min. 1.28 and max 7.7, corresponding to min. 1.61 and max. 11.9 g/kg DM. Regession analysis show significant negative relationships between TP and potassium (K, mean 32, min. 11, max. 50 g/kg DM; TP, % total-N=66-0.5K, g/kg DM, r=0.4) and K vs. Glu, whereas increasing concentrations in silage K correspond to increasing concentrations of GABA (GABA, g/16 N=0.95+0.062K, g/kg DM, r=0.51).

Tab.1: Chemical data on grass silages

		n	mean	SD		mean	SD
pH		95	5.3	0.5	Met+Cys[3)]	1.86	0.34
Lactic acid	mmol/kg [1)]	94	51.3	40.4	Lys	3.48	0.67
Acetate	mmol/kg	97	92.9	52.6	Thr	3.23	0.64
Propionate	mmol/kg	97	15.1	16.7	Arg	2.42	0.72
iso-Butyrate	mmol/kg	97	5.6	9.1	Ile	3.63	0.47
n-Butyrate	mmol/kg	97	62.1	76.3	Leu	6.46	0.79
iso-Valeriate	mmol/kg	97	3.9	8.4	Val	5.15	0.53
n-Valeriate	mmol/kg	97	2.7	5.1	His	1.60	0.27
Dry Matter (DM)	g/kg	132	438	106	Phe	4.24	0.53
Crude Ash	g/kg DM	131	106	23	Gly	4.16	0.57
Crude Fibre	g/kg DM	131	194	32	Ser	2.91	0.57
Crude Protein (CP[2)])	g/kg DM	131	268	26	Pro	5.55	1.51
Ammonia-Nitrogen	% of total N	30	7.9	1.4	Ala	5.92	0.93
True Protein (TP)	g/kg DM	94	97	21	Asp	7.82	1.56
	% of CP	94	49	11	Glu	6.84	1.19
[1)] as fed; [2)] Nx6.25; [3)] g/16 N, n=85, GABA n=80					GABA	2.94	1.02

Conclusion: The degree in proteolysis of grass protein during conservation is enforced in grass with high K-concentrations. None of the other analytes showed a link to the variation in the CP-TP-ratio. The chemical nature of the non-protein-N is not completely defined. Free AA and amines contribute to this fraction but in the silages presented here common biogenic amines (data not shown) did not scatter in relation to change in TP. In contrast, the loss in protein corresponds to a loss in Glu and an increase in GABA. Obviously GABA has no potential to depresst DM intake in ruminants (1,2) but may ameliorate responses on heat stress (2) in cows ingesting GABA by 40 mg/kg DM. However, the GABA ingestion in dairy cows can exceed 50 g/d (>70 mg/kg BW). The hypothesis is that a high uptake of GABA may interact with endocrine responses i.a. those regulating gluconeogenesis (3). If so, high concentrations of GABA in silages should be considered as undesirable related herd health. Subsequent studies are directed to the ruminal bypass of GABA and postprandial blood levels.

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154. Excretion of nitrogen compounds by growing goats fed diets containing different levels of lucerne hay, compound feed and produce

Einfluss der Fütterung gemischter Rationen aus Luzerneheu und Konzentratfutter mit Saftfutteranteil auf die Ausscheidung N-haltiger Verbindungen wachsender Ziegen

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Question: Rations fed to ruminants with high amounts of rapidly fermentable carbohydrates might lead to rumen acidosis and alter nitrogen excretion. Especially sugar-rich feedstuffs like fruits and vegetables (produce) are a matter of interest in this respect as domestic and wild ruminants in zoos are commonly fed with considerable amounts of produce. The hypothesis of this study was that feeding diets with different levels of produce and compound feed as concentrates combined with two lucerne hays of different quality affects excretion of nitrogen compounds via urine and faeces. To correspond at most with grazing domestic as well as browsing zoo ruminants, goats were taken as model animal due to their intermediate feeding type characteristics.

Methods: Twelve growing goats were assigned in two groups of six animals each and received lucerne hay of intended high quality (HQ) or low quality (LQ) as forage. A mixture of apples, carrots and celery (1/3 of each in fresh matter) was used as produce and mixed with compound feed at a ratio of 10:90 on a dry matter (DM) basis. The mixture of produce and compound feed was combined with lucerne hay of HQ and LQ at ratios of 50:50 and 30:70, respectively, in a 2 x 2 factorial design resulting in six observations per treatment. Animals were adapted to their respective ration for 9 days followed by quantitative sampling of urine and faeces in metabolic cages for five days. Body weight was determined before and after sampling periods. Chemical analysis (crude protein [CP], ash, crude fat) was done on all diet components. Urine and faeces were analyzed for Kjeldahl nitrogen (N) and for urea and NH_3 (photometric test) or purine derivatives (HPLC). Data was analyzed with a mixed model with repeated measures for goats and ratio, hay quality as well as sampling period as fixed factors (level of significance $p \leq 0.05$).

Results: Surprisingly LQ hay contained more crude protein than HQ hay (178 and 167 g/kg CP, respectively), although signs of quality like structure, proportion of leaves and color indicated a distinctly higher quality in the HQ hay. Initial and final body weight were higher in the first sampling period than in the second ($P < 0.001$), whereby body weight gain during sampling periods was not affected. The N balance (mean ± SE) is shown in the Table.

	HQ		LQ	
	50:50	30:70	50:50	30:70
N-Intake, g/d	32.9 ± 0.1	32.0 ± 0.2	34.1 ± 0.3	33.6 ± 0.3
N-Excretion				
Urine, g/d	20.9 ± 1.1	20.5 ± 1.2	19.2 ± 0.6	19.2 ± 0.7
Faeces, g/d	9.6 ± 0.3	10.0 ± 0.7	11.3 ± 0.7	12.0 ± 0.2
N-Retention, g/d	2.6 ± 1.0	1.5 ± 1.4	3.6 ± 0.7	2.4 ± 0.5

Urine volume, urine-N, urea-N and NH_3-N differed between sampling periods (all $P < 0.001$). In diets with 50:50 ratio urinary NH_3-N ($P < 0.05$) and xanthine ($P < 0.05$) was higher than in diets with a 30:70 ratio Urinary hippuric acid excretion was higher in diets with hay of HQ than LQ ($P < 0.001$), whereas faecal N was higher in diets with hay of LQ than HQ ($P < 0.05$). Proportions of faecal N from undigested forage and metabolic N of total faecal N were similar, whereas proportion of bacterial N was higher in HQ hay ($P < 0.05$) and with a ratio 30:70 ($P < 0.05$).

Conclusion: Faecal N excretion followed hay quality presumably due to differences in forage digestibility. Higher amounts of produce and compound feed in the ration led to a greater N retention, resulting from higher contents of easily digestible carbohydrates or rather metabolizable energy. Evidences for rumen acidosis caused by the proportion of produce in diet were not observed, certainly due to the relatively restrictive use of fruits and vegetables in the present diets. For clearer evidence of possible negative influences of high amounts of produce on rumen physiology, trials must be conducted with considerably higher amounts of fruits and vegetables in the diet. Until then *in vitro* studies can give more valuable information on the suitability of produce as feed for ruminants.

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155. Permeation of short-chain fatty acids (SCFA) across the ovine reticular epithelium

Permeation kurzkettiger Fettsäuren (SCFA) über das Haubenepithel des Schafes

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Introduction: Short-chain fatty acids (SCFA) produced by microbial fermentation of carbohydrates in the forestomach form the basis for ruminant´s energy maintenance. The majority of SCFA is taken up directly from the reticulorumen. While the mechanisms of SCFA-absorption across the ruminal wall were in focus of research in the last decades, less is known about the SCFA absorption in the second part of the forestomach, the reticulum.

Methods: Epithelia were obtained from female merino breed sheep of different age which were fed with hay and water ad libitum for at least two weeks prior to the experiments. Stripped epithelia of ovine reticulum were mounted in Ussing-chambers under short-circuit conditions. Fluxes of radiolabelled acetate or butyrate across the reticular epithelium were measured in mucosal to serosal (Jms) and serosal to mucosal direction (Jsm). EIPA was used to inhibit Na-H-exchanger; pHMB was applied as inhibitor of monocarboxylate transporters (MCTs); fenoprofen was used as inhibitor of sodium-coupled monocarboxylate-transporters (SMCTs); the Na-K-ATPase was disabled by ouabain. Electrophysiological parameters were monitored throughout the experiment. Statistical evaluation is based on the number of animals used.

Results: For butyrate, Jms was significantly higher than Jsm (1.87 ± 0.35and 1.12 ± 0.12 µmol cm^{-2} h^{-1}, respectively, $p = 0.013$, $n = 8$; net absorption). For acetate, Jms was nominally lower than Jsm but this difference did not reach significance level ($p = 0.13$). Hence, no net flux of acetate was observed. Jms of butyrate was approximately twice as high as that of acetate. Incubation with ouabain abolished short-circuit current and reduced Jms of butyrate by about 32% (0.69 ± 0.19 µmol cm^{-2} h^{-1}, $n = 8$, $p < 0.001$). Jsm of butyrate was not affected by ouabain treatment. pHMB reduced Jms of butyrate from 2.02 ± 0.4 to 1.29 ± 0.22 µmol cm^{-2} h^{-1} ($n = 8$, $p = 0.005$) but did not affect Jsm of butyrate. Both Jms and Jsm of butyrate were reduced in tendency by fenoprofen application (about 10 % reduction, $p = 0.05$, $n = 8$). EIPA did not affect Jms of butyrate but reduced Jsm of butyrate by about 11% ($p = 0.018$).

Conclusion: Our results show that SCFA (at least butyrate) are effectively taken up by reticular epithelium. The mechanisms behind are at least partially sodium and/or voltage dependent. The results of our inhibitor studies show that MCTs and SMCTs could be involved in the permeation of butyrate across the reticular epithelium.

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156. Effect of dietary nitrogen and calcium on intestinal calcium absorption and on tight junction protein expression in young goats

Beeinflussung der intestinalen Calciumabsorption und Expression von Tight Junction-Proteinen durch die diätetische Stickstoff- und Calciumversorgung bei wachsenden Ziegen

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Feeding a nitrogen- (N) reduced or a combined N- and calcium- (Ca) reduced diet to ruminants lead to a decrease of plasma calcitriol concentrations (1). In monogastric species calcitriol is able to enhance paracellular Ca absorption (2), which is mainly mediated by tight junction (TJ) proteins. For small ruminants, the expression of intestinal TJ proteins has not been studied yet. Therefore, the expression of TJ proteins in caprine intestinal epithelia was characterized and the effect of a dietary N- and/or Ca-reduction on their RNA and protein expression was investigated.

Animals and Methods: Four groups of male German colored goats (n = 6-7) received either a control (N+/Ca+), reduced N (N-/Ca+), reduced Ca (N+/Ca-) or reduced N and Ca diet (N-/Ca-) for at least six weeks. Diets were isoenergetic, containing 11.2 MJ ME/kg DM. Calcitriol plasma concentrations were measured. Unidirectional flux rates of Ca were determined in Ussing chambers. Expression levels of mRNA and protein of vitamin D receptor (VDR), claudin 2 and 12, occludin, cadherin 17 and zonula occludens protein 1 (ZO 1) were determined by *q*PCR and Western Blot analyses. Data were analyzed by two-way ANOVA and Pearson's correlation.

Results: Goats on the (N-/Ca+) diet showed 32% lower, goats on the (N+/Ca-) diet 93% higher plasma calcitriol concentrations compared to the control group. Intestinal net flux rates of Ca were increased by dietary Ca-reduction but decreased by N-reduction and by N- and Ca-reduction. Expression of Cld 2 and 12, occludin, Cdh 17 and ZO 1 in caprine intestinal epithelia could be demonstrated for the first time. While neither VDR nor Cdh 17 expression was affected by the feeding regime, the reduced dietary N-supply led to an increased expression of occludin on RNA level as well as of ZO 1 on RNA and protein level. RNA expression of Cld 2 was elevated due to a dietary Ca-reduction, whereas occludin expression was diminished on RNA and protein level by this feeding regime. The RNA expression of Cld 2 showed a positive correlation ($P < 0.05$), the expression of occludin a negative correlation ($P < 0.05$) with plasma calcitriol concentrations.

Conclusions: A dietary Ca-reduction led to an increased intestinal Ca-absorption, partly based on enhanced paracellular transport capacity, characterized by an increased expression of TJ proteins mediating paracellular Ca-permeability. Correlation between plasma calcitriol concentrations and the expression of Cld 2 and occludin implicatedthat in ruminants like in monogastric species, calcitriol might play an important role in the regulation of paracellular Ca absorption. The lack of these effects when dietary Ca was reduced in combination with a reduced N supply was probably caused by decreased plasma calcitriol concentrations.

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157. Effects of a ration change from TMR to pasture on rumen parameters of dairy cows

Effekte der Umstellung von einer TMR- auf eine weidebasierte Fütterung auf Pansenparameter von Milchkühen

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In pasture-based dairy production systems, dairy cows often receive a silage- and concentrate-based ration (=TMR, total mixed ration) during wintertime and are gradually introduced to fresh herbage in spring. The present study aims to investigate how the rumen total content, mean papillae area, and buffering capacity are affected by this nutritional change.

Methods: A ten week trial (w1-10) was performed in the spring of 2014, involving ten rumen-fistulated dairy cows of the German Holstein breed (182 (s.d. 24) days in milk, 23.5 (s.d. 3.5) kg milk/day). The cows were divided into a pasture- and a confinement group (PG and CG, n=5). The CG stayed on a TMR-based ration (35% corn silage, 35% grass silage, 30% concentrate; DM basis), while the PG was gradually transitioned from a TMR- to a pasture-based ration (w1: TMR-only, w2: 3h/day on pasture, w3&4: 12h/day on pasture, w5-10: pasture-only). In w1, w5, and w10 the total liquid and solid rumen content were separated and weighed, and rumen papillae biopsies were taken at three locations within the rumen. Subsequently, a "short chain fatty acid - absorption test (SCFA-AT)" was performed: a buffer (31.5L (s.d. 0.4), pH 5.0 (s.d. 0.1), 400mOsm/L) containing SCFA (170mM, 60% acetic, 25% propionic, 15% butyric acid), salts and a marker (Co-EDTA, 0.07g/L) was introduced into the emptied and washed rumen. Immediately after introduction and after 60min, buffer samples were collected and the pH was measured every 15min. The papillae were photographed and the surface area was determined using CellProfiler®. Data were analysed as repeated measures using PROC MIXED of SAS Enterprise Guide 4.3® with week and diet group and their interaction as fixed factors and cow as random factor.

Results: The rumen solid content, but not the liquid content, decreased and the liquid/solid-ratio increased in the PG in w5, most likely due to a lower DM intake. Concurrently a lower end pH during the SCFA-AT, indicating a lower rumen buffering capacity, as well as a decreased mean papillae area was observed. In w10 these parameters approached the level of the CG again.

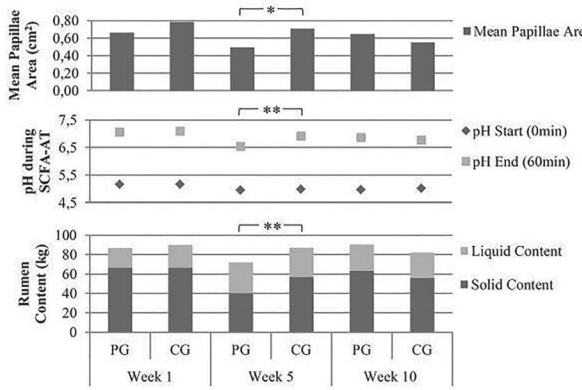

Fig.1: Mean rumen papillae area, pH during SCFA-AT and total rumen content at three points within the trial (means, n=5). PG=pasture group, CG=confinement group, *= P<0.05, **= P≤0.01.

Conclusion: During the transition from a TMR-to a pasture-based ration, the solid rumen content, the mean papillae area, and the end pH during the SCFA-AT decreased. Analysis of the buffer samples collected during the SCFA-AT is needed to identify whether the change in rumen buffering capacity is mediated by a lower SCFA-absorption rate and whether there is a correlation with the observed decreased papillae surface area.

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158. Ruminal short-chain fatty acid absorption in response to long-term SARA induction

Absorption der kurzkettigen Fettsäuren im Pansen in Abhängigkeit einer induzierten SARA

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Subacute ruminal acidosis (SARA) is characterized by a decrease of ruminal pH to a certain threshold, e.g. pH < 5.8 for 3-5h/d (1). Greater absorption of short-chain fatty acids (SCFA) produced from fermentation of diets helps to stabilize ruminal pH, thus the absorption efficiency during SARA is likely to be different from that of normal ruminal conditions. However, most of the previous findings were based on short-term SARA induction periods of 1-2 wk. Obviously, this does not represent the field situation. Also, such short periods might not be sufficient for maximal rumen papillae adaptation (2). In this study we investigated SCFA absorption in response to normal and SARA conditions with two different long-term (4 wk) SARA-challenge models.

Methods: The experiment followed a 2x2 crossover design with 2 SARA-challenge models (transient and persistent SARA) and 2 experimental runs. Eight rumen-fistulated non-lactating Holstein cows were randomly assigned to one of the two SARA models (n=8). At the start cows were based on a 100% forage diet (baseline), for the next 7 d they were gradually adapted to a high grain ration with the target of 60% concentrate of DM intake, leading to the onset of SARA as determined by ruminal pH using wireless sensors (1). The high grain feeding, and thus the SARA condition, was maintained for the next 28 d for the persistent model. For the transient model, SARA was interrupted by feeding forage-only from d8 to d14, followed by re-induction of SARA (with 2 d of adaptation) for the next 12 d. Subsequently, 100% forage was again fed to all cows for 8 wk (wash-out period) until the next run. SCFA absorption was determined by the washed reticulorumen procedure (WRP) (3) which was carried out at different time points: (i) baseline, (ii) the last day of SARA challenge period, and (iii) 2-3 wk of the wash-out (i.e. recovery) period. Experimental buffer of WRP with known concentrations of SCFA (acetate, propionate, and butyrate) was sampled at 0, 35, 65 min after application for the analysis of SCFA absorption rates. Data were subjected to statistical analysis using Proc Mixed of SAS with the model including the fixed effects of SARA model, feeding period and their interaction, experimental run, and sequence (carryover effect) and the random effect of cows nested within sequence.

Results: The 8-wk wash-out period was sufficient as there was neither carryover nor run effects. No effect of SARA model, feeding period and their interaction was observed on most SCFA absorption parameters. On average, absorption rate of total SCFA at 35 min of WRP at baseline, SARA and recovery was 692, 1003 and 752 mmol/h, respectively and the fractional absorption rate was 30, 44 and 40%/h, respectively but there were large variations among replicates, also true for individual SCFA parameters. Despite such considerable variations, there was an interaction effect on the absorption rates of acetate ($P<0.05$) and a trend for fractional absorption rate of total SCFA ($P=0.099$) during the last 30 min. The transient model resulted in increased absorption rates of acetate during the SARA challenge compared to baseline (~1.5 fold) and recovery (~2.5 fold) while the persistent SARA showed similar values across periods.

Conclusions: The greater acetate absorption rate during SARA found with the transient SARA but not with the persistent SARA model suggests that there might be differences in morphological or functional adaptation of the ruminal epithelium between the two models with potential consequences for the severity of SARA.

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159. Effects of experimentally induced pancreatic exocrine insufficiency in young pigs on morphometry and function of the small intestines

Einfluss einer experimentell induzierten exokrinen Pankreasinsuffizienz auf die Morphologie und Funktion des Dünndarmes

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In former studies [1] an elongation of the small intestines was found in piglets with experimentally induced pancreatic exocrine insufficiency (PEI). Ussing chamber studies under short circuit current (Isc) conditions revealed that electrogenic transport of glucose was increased in the jejunum in pigs with pancreatic duct ligation at week 7 of life [1], accompanied by enhanced jejunal tissue conductance (Gt) and permeability [1; 2]. The aim of this study was to examine the impact of pancreatic duct ligation (PL) in pigs that underwent surgery at different age (week 7 or 16 of life) on body morphology and active transport features in the small intestines.

Methods: Overall 22 piglets were used in this study. In 8 pigs the PL surgery was performed at week 7 (PL 7) while 7 pigs underwent surgery at an age of 16 weeks (PL 16). None of the PL pigs was treated with pancreatic enzymes. Control pigs (n=7) underwent sham OP at the age of 7 weeks. A complete diet (% of dry matter: 37.3 starch, 11.5 crude fat and 20.3 crude protein) was fed (pair feeding; last two weeks: ad libitum feeding). All animals were euthanized at the age of 26 weeks and dissected. Defined small intestinal segments were used for Ussing chamber studies regarding active transport of glucose and dipeptides as well as protein sampling for Western blots.

Results: Body weight in week 26 was markedly reduced by PEI and also affected by the age of the pigs at surgery (more distinct reduction in PL 7). The small intestines were significantly elongated in PL 16 compared to controls (table 1). Jejunal Gt and glucose induced Isc were increased (p+/Glucose cotransporter SGLT1 revealed a significant increase in the jejunum of PL 7 pigs compared to control group.

Table 1:Body weight, body length, small intestine length and functional features of jejunum and ileum of control pigs and pancreatic duct ligated pigs - measured at the age of 26 weeks

	Control (n=7)	PL 7 (n=8)	PL 16 (n=7)
Body weight (kg)	118 ± 8.50a	49.7 ± 23.7c	96.2 ± 10.7b
Length (nose to tail; m)	1.42 ± 0.06a	1.14 ± 0.14 c	1.36 ± 0.06b
Small intestines length (m)	18.5 ± 1.49a	21.1 ± 3.21ab	24.7 ± 2.47b
Gt (jejunum) $mS*cm^{-2}$	28.8 ± 5.8a	40.1 ± 7.7b	44.0 ± 7.7b
Gt (ileum) $mS*cm^{-2}$	25.9 ± 6.2a	27.8 ± 7.9a	23.7 ± 6.9a
Jejunal ΔIsc Gly-Gln ($\mu eq*cm^{-2}*h^{-1}$)	0.13 ± 0.09a	0.89 ± 0.75b	0.47 ± 0.24ab
Jejunal ΔIsc Glucose ($\mu eq*cm^{-2}*h^{-1}$)	0.18 ± 0.13a	2.05 ± 1.42b	1.44 ± 0.52b
Ileal ΔIsc Gly-Gln ($\mu eq*cm^{-2}*h^{-1}$)	0.29 ± 0.17a	0.16 ± 0.20a	0.05 ± 0.04b
Ileal ΔIsc Glucose ($\mu eq*cm^{-2}*h^{-1}$)	2.46 ± 1.11a	2.71 ± 4.14a	0.52 ± 0.75b
Jejunal SGLT1 expression*	0.40 ± 0.19a (N=3)	0.96 ± 0.23b (N=5)	0.45 ± 0.12a (N=5)
Ileal SGLT1 expression*	0.20 ± 0.06a (N=3)	0.35 ± 0.14a (N=5)	0.22 ± 0.07a (N=5)

* (β-actin norm. units); different letters mark significant effect of group ($p<0.05$)

Conclusions: Experimentally induced PEI resulted in morphological (elongation of the small intestine) and functional changes (increased *in vitro* glucose and dipeptide transport of the jejunum). These changes were also reflected at transporter level and are assumed to be an adaption to reduced nutrient availability. Interestingly the ileal ΔIsc after addition of dipeptides or glucose was lowest while small intestine length was highest in PL 16. Significantly higher jejunal SGLT1 expression in PL 7 indicate that compensatory changes in mucosal transporter abundance induced by modified digestive processes seem to be more pronounced in this intestinal segment.

References: available on request

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160. Formula compared to sow milk feeding changes digestive physiology and microbial ecology in the small intestine of new-born piglets

Formulafütterung gegenüber Sauenmilch verändert die Verdauungsphysiologie und mikrobielle Ökologie im Dünndarm von neugeborenen Ferkeln

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Question: Selection for increased litter size in swine industry has resulted in an increased number of life born piglets per litter and piglets with low birth weight (1) with concomitant negative outcomes on piglet survival and sow longevity. Recent developments to improve the situation include artificial rearing programs with cow milk based formula feeding. Existing information about intestinal development in formula fed pigs are to date mainly based on experiments in preterm piglet models (2). As there is limited information available on the development of the intestinal tract of vaginally delivered young piglets subjected to artificial rearing and formula feeding, we studied the effect of formula feeding on various aspects of intestinal physiology and microbial ecology development.

Methods: Sixteen new-born piglets were used in this study. They were either removed within 4h from their mothers and fed a formula based on skimmed milk powder and whey from cow milk (22.6% CP, 20.0% EE, 46.0% lactose per kg DM), or suckled by their mothers together with remaining littermates (average milk composition: 30.2% CP, 37.3% EE, 21.0% lactose per kg DM). After two weeks, eight piglets per treatment were killed and jejunal contents and tissues sampled. Brush border membrane disaccharidase activity (lactase, sucrase, maltase) and glucose transport capacity (in Ussing chambers) was determined in the tissue samples. Gene expression of lactase-phlorizin hydrolase (LPH) and Na-dependent glucose transporter 1 (SGLT-1) was determined. Major bacterial groups and *E.coli* pathogenic factors were analysed by qPCR and multiplex PCR, respectively. Microbial metabolites including D/L-lactate (high-performance liquid chromatography), SCFA (gas chromatography), NH_3 (colorimetrically) and biogenic amines (ion-exchange chromatography) were determined in jejunal contents. Means were compared by ANOVA procedures and subsequent Tukey HSD test using SPSS (version 21.0, Chicago, USA).

Results: No significant differences were observed for daily weight gain and final body weight, but formula fed pigs tended ($P < 0.1$) to gain less and were lighter at the end of the experimental period. Intestinal lactase activity tended to be higher in suckled pigs whereas maltase activity was lower. Na-dependent glucose transport and expression of SGLT1 was higher ($P<0.05$) in formula fed pigs, whereas LPH expression was not different between groups. The 16S rRNA gene copy numbers of lactobacilli, bifidobacteria, enterococci, bacteroides, clostridial cluster I, IV and XIVa were lower ($P<0.05$) in formula fed piglets. Similarly, concentration of acetate, butyrate and propionate were lower ($P<0.05$) in formula fed piglets, whereas lactate and NH_3 were unaffected. *E.coli* fimbriae and toxin genes (fae, fedA, estIb) were detected only occasionally in both groups.

Conclusions: Differences in jejunal enzyme activity, sodium-dependent glucose transport capacity and gene expression between suckling and formula fed piglets cannot solely be explained by substrate availability in milk or formula, respectively. The changes seem to be accompanied with a delayed establishment of a functional microbial ecosystem, likely due to a lack of contact with the mother and the surrounding environment as compared to suckling piglets. Whether formula feeding may predispose the new-born piglet to intestinal infections and dysfunction during the pre- and post-weaning period needs further clarification.

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161. Is equine jejunal glucose transport modified by ischemia?

Wird der Transport von Glukose im equinen Jejunumepithel durch Ischämie beeinflusst?

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Introduction: The equine jejunum is often affected by ischemia. Because of its wide mesentery suspension, dislocations and resulting occlusions of the jejunal lumen as well as supplying mesenterial vessels can easily arise. This leads to impairment of important intestinal functions, e.g. motility, permeability to bacteria and clearance of toxic substances. However, the effects of ischemia on the pivotal function of the jejunum, the absorption of nutrients, are still not completely uncovered.

Aim: Thus, our study aimed at investigating the effects of ischemia on the transepithelial transport of glucose in equine jejunal epithelium.

Methods: We used jejunal epithelia of freshly euthanized horses and mounted them in Ussing chambers after stripping off the muscle layers. After an equilibration period we measured fluxes of 3 mM (radioactively labelled) D-glucose (G), 3-ortho-methyl-glucose (OMG) (N = 3; n = 6) or 10 mM mannitol (M) across the epithelium (N = 6; n = 12). In another group of epithelia, we added 2 mM glucose to the (glucose-free) mucosal buffer solution repeatedly and measured the resulting increase in short circuit current (ΔI_{sc}) (N = 7; n = 14). All epithelia were gassed with 100% oxygen at the beginning, then part of the epithelia was switched to gassing with 1% oxygen and 99% nitrogen after the first flux period or the first addition of glucose, respectively, for one hour ("ischemia"). After that, epithelia were gassed with 100% oxygen again for two more hours. Another group of epithelia was gassed with 100% oxygen for the whole incubation period and served as a control group.

Results: We observed a significant decrease in ΔI_{sc} under "ischemia" compared to the control group gassed with 100% oxygen ($p < 0.05$, paired t-test). This decrease did not recover while gassing with 100% oxygen again after one hour. Fluxes of M and OMG did not differ between the different gassing regimes. Fluxes of G, however, were increased significantly by "ischemia" compared to the control group ($p < 0.05$, paired t-test).

Conclusions: Our results regarding the electrogenic transport of glucose depicted by ΔI_{sc} point to a decrease in Na^+-coupled glucose transport via SGLT 1 during ischemia. This seems to be a sensible reaction in order to save ATP for other vitally important processes. In contrast to these findings are the increased flux rates of G, indicating an increased involvement of other, non-electrogenic transport proteins in the transport of glucose. As fluxes of M were not affected by "ischemia", there seems to be no change in paracellular conductance. A candidate for non-electrogenic transepithelial glucose transport is glucose transporter 2 (GLUT 2), which is known to be recruited to the apical membrane under high glucose loads[1]. However, GLUT 2 recruitment would also result in an enhanced transport of OMG, thus casting this hypothesis into doubt, but hinting at metabolic effects on glucose transport. In future studies, we will try to investigate the role of GLUT 2 on the one hand and that of glucose metabolism on the other in ischemic equine jejunal epithelium further.

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162. Mechanisms of Ca^{2+} and P_i transport in equine small intestines

Mechanismen des Ca2+- und Pi- Transportes entlang des equinen Dünndarmes

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In vivo as well as *in vitro* studies demonstrated that the small intestines contribute to a larger extent to overall calcium (Ca^{2+}) absorption than the hindgut. In respect to phosphate (P_i), a secretion could be shown in upper jejunum while P_i absorption was revealed in hindgut (1, 2). The aim of the current study was to investigate basic characteristics of Ca^{2+} and P_i transport systems by means of Ussing chamber technique. Mannitol flux rates and inhibitors were used to differentiate between paracellular and transcellular transport.

Animals and Methods: Intestinal tissues from 12 healthy, mature horses aged two to 22 years were included in the study. Duodenal and jejunal epithelia were incubated in Ussing chambers in absence of any electrochemical gradient for determination of unidirectional flux rates of Ca^{2+}, P_i and mannitol. Differentiation between paracellular and transcellular transport was done by correlation of unidirectional Ca^{2+} and P_i flux rates with corresponding mannitol flux rates (Pearson correlation coefficient test followed by linear regression analysis in case of significance). Further characterization of P_i transport was carried out by application of phosphonoformic acid (PFA) and sodium arsenate, chemicals which have been shown to inhibit sodium dependent P_i transporters in other species. Significance was set at $P<0.05$.

Results: In the duodenum, Ca^{2+} flux rates from the serosal to the mucosal side of the epithelium (J_{sm}) showed a positive correlation with J_{sm} of mannitol. Flux rates of Ca^{2+} from the mucosal to the serosal side (J_{ms}) exceeded J_{sm} resulting in a positive net flux. No correlation could be revealed for J_{ms} of Ca^{2+} and mannitol. In the jejunum, a negative net flux could be found for P_i. While a significant correlation was detected between jejunal J_{ms} of P_i and mannitol, J_{sm} flux rates of P_i were independent from respective J_{sm} of mannitol. Addition of PFA or sodium arsenate had no significant effect on P_i flux rates.

Conclusions: The lack of linear correlations between J_{ms} of mannitol and Ca^{2+} supposed a predominantly transcellular transport of Ca^{2+} in the duodenum as well as in the jejunum. In contrast the secretion of P_i in the small intestine is most probably mediated by active transcellular mechanisms. The resulting increase in luminal P_i concentrations *in vivo* might be of importance for the microbial community of the hindgut.

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163. Effects of individual short chain fatty acids on tight junctions of the ovine ruminal epithelium

Wirkung einzelner kurzkettiger Fettsäuren auf die Tight junctions im Pansenepithel von Schafen

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Short chain fatty acids (SCFA) are the first source of energy for ruminants. Such acids result from the microbial catabolism of carbohydrates in the forestomach (1). In domestic species, acids released in the rumen milieu can reach high concentrations. Albeit SCFA are an effective source of energy for ruminant animals, an overproduction may be deleterious. The three most abundant SCFA (acetate, propionate and butyrate) are normally produced within a flexible range of concentrations and their amount varies significantly in response to different diets and rumen pH (2).The central question of the present study was whether individual SCFA are able to affect the barrier function of the ruminal epithelium of sheep.

Methods: Ruminal epithelial preparations were mounted in Ussing chambers. The buffer solution on the serosal side had a pH of 7.4, was SCFA-free but contained glucose (10 mM) and L-glutamine (1 mM) for the nutrition of the epithelium. At the mucosal side, buffer solutions with pH 6.1 were applied that contained either no SCFA (control), 30 mM acetate, 30 mM propionate, or 30 mM butyrate. As acetate can reach the highest concentration in the rumen, an additional group with 100 mM mucosal acetate was included. After 7 h of incubation, tissues were removed from the chambers. As the barrier function aligns with expression of tight junction proteins in sheep ruminal epithelium (3), mRNA and protein expression of claudins-1, -4, -7, and occludin was analyzed, using quantitative real-time PCR and Western blot techniques, respectively. Data are means ± SEM. Statistical comparisons of groups was performed by one way analysis of variance (ANOVA) with posthoc comparisons versus control (Dunnett´s method) using the statistical software SigmaPlot 11.0.

Results: Tissue conductance increased by 71 ± 25% ($P < 0.05$) when epithelia were incubated with 30 mM butyrate on the mucosal side over 7 h, while other SCFA had no effect on tissue conductance. The mRNA and protein expression of occludin was not changed, but mRNA expression of claudin-1 increased with 100 mM acetate (by 128 ± 33%; $P < 0.05$), 30 mM propionate (by 189 ± 65% SEM, $P < 0.05$), and 30 mM butyrate (by 58 ± 8%; $P < 0.05$). In contrast to increased mRNA expression, protein expression of claudin-1 was not changed by 30 mM or 100 mM acetate, as well as 30 mM propionate, but was decreased with 30 mM butyrate (by 41 ± 11%; $P < 0.05$). The mRNA expression of claudin-4 was increased after incubation with 30 mM propionate (by 153 ± 23%; $P < 0.05$) and 30 mM butyrate (by 152 ± 28%; $P < 0.05$). However, none of the treatments decreased the expression of claudin-4 protein. Incubation with different SCFA did not significantly change claudin-7 mRNA expression. However, claudin-7 protein was down-regulated after incubation with 30 mM butyrate for 7 h (by 66 ± 13%; $P < 0.05$).

Conclusion: High concentrations of butyrate impair the paracellular barrier function of ovine ruminal epithelium under physiological pH conditions *in vitro*. The effect is evident from an increased tissue conductance and decreased claudin-1 and claudin-7 protein expression. The impairment occurs despite a stimulation of the mRNA synthesis of claudin-1 and claudin-4. Acetate and propionate appear to have no significant effect on ruminal barrier function under these experimental conditions.

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164. Effects of an acidogenic, grain-rich diet on short chain fatty acids absorption from the washed reticulorumen procedure of cows

Auswirkungen einer azidogenen, Getreide-reichen Ration auf die Resorption kurzkettiger Fettsäuren aus dem gewaschenen Retikulorumen von Kühen

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Rapidly fermentable carbohydrates are usually fed to dairy cows to achieve high productivity. Carbohydrates are converted into short chain fatty acids (SCFA) as a result of end products of microbial fermentation in the rumen and provide up to 80% of metabolizable energy. When SCFA absorption is less than production, there is a risk of sub-acute rumen acidosis (SARA). Gradual transition from low to higher fermentable diet could decrease this metabolic disorder, e.g., by stimulating the cellular proliferation which provides more surface area for SCFA absorption. The current study was undertaken to reveal the absorption of SCFA at different feeding periods before, during and after a grain-induced SARA.

Methods: A feeding trial was conducted with eight non-lactating rumen-cannulated Holstein cows in two sequential runs (n=8); each run lasted for 5 wks. Cows were initially fed only a forage-mix diet (baseline). After baseline, cows were gradually transitioned to 60:40 ratio of grains to forage mix over a 7-d period, and SARA was continued for 1 wk followed by 1 wk of forage only. Afterwards, SARA was re-induced for 12 d with a 2-d adaptation. Finally, the cows were returned to the forage diet for 2 wks of recovery from SARA. Indwelling wireless sensors were used continuously throughout the experiment to measure pH, and to confirm SARA (1). The washed reticulorumen procedure (WRP) was performed (2) with each cow at 4 time points: baseline, gradual d-7 of SARA induction, the end of re-induction and 2 wk of recovery period. During WRP, digesta was removed from reticulorumen and cleaned with washing buffer. Afterwards, 20 L experimental buffer containing CaCl2 (2 m*M*), MgCl2 (2 m*M*), NaCl (10 m*M*), sodium acetate (60 m*M*), K2HPO4 (5 m*M*), sodium propionate (30 m*M*), butyric acid (10 m*M*), D/L-lactic acid (5 m*M*), NaHCO3 (25 m*M*), lactulose (5 m*M*) and Cr-EDTA (1.8 m*M*) was infused into the reticulorumen of cows for 65 minutes. Experimental buffer samples were collected at 0, 35 and 65 min from reticulorumen for SCFA absorption analysis by gas chromatography. Data was analyzed by PROC MIXED procedure of SAS to test the effect of the feeding period.

Results: The absorption of SCFA during the first 35 min (F35) and the last 30 min (L30) were examined. Absolute absorption rate (mmol/h) and fractional absorption rate (%/h) of individual SCFA (acetate, propionate and butyrate) and total SCFA during F35 were not affected by feeding period. Nevertheless, an effect of feeding period was found during L30 of acetate absorption rates ($P<0.05$). Absolute and fractional absorption rates were highest during re-induction of SARA (620.4 mmol/h and 59.0%/h), lowest during the recovery period (223.8 mmol/h and 24.4%/h), while baseline and d-7 gradual period of SARA induction showed intermediate values. A similar trend was found for the absolute total SCFA absorption rate ($P=0.10$) but not for the fractional rate of total SCFA absorption. Both absolute and fractional absorption rates of propionate and butyrate were not affected by feeding period.

Conclusions: An increased SCFA absorption was evident only during the re-induction period. This suggests that adaptation of the rumen papillae to enhance the absorptive surface area as a means to increase SCFA absorption requires time (longer than 1 wk) to overcome high production of SCFA during high grain feeding.

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165. Dynamic regulation of tight junction protein localization in murine mammary gland epithelium after discontinued suckling

Dynamische Regulation von Tight Junction-Proteinen im Brustdrüsenepithel

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Outline: Milk secretion and composition is closely related to the barrier function provided by tight junction (TJ) proteins of the mammary gland epithelium (1). Recently, claudins could be identified to primarily determine paracellular permeability in different organs, and were categorized into three groups, namely (i) claudins providing a paracellular pore function, (ii) claudins tightening tight junctions, and (iii) claudins with ambiguous functions (2). Aside from direct barrier formation, additional functions including mediation of adhesion are currently discussed. For analysis of a possible role of single members of the claudin family for additional functions, mammary gland tissue specimens obtained from the mouse model of discontinued suckling were analyzed.

Methods: Tissue specimens were obtained from experiments employing the mouse model of 20 h discontinued suckling as described in detail recently (3). Briefly, tissues were fixed, and paraffin-embedded; morphometrical characteristics were analyzed in cross sections, and immunostaining was performed with antibodies raised against single members of the claudin family and occludin. Localization was analyzed employing a fluorescence microscope and laser-confocal imaging of immunofluorescent stainings. Statistical analysis was performed using Student's t test. $P < 0.05$ was considered significant.

Results: 20 h interruption of suckling resulted in an increase of alveolar size, and a flattening of epithelial cells ($p<0.001$, $n = 5$ and 6, respectively). Mammary epithelial cells lining alveoli revealed increased signals of claudin-1 and claudin-3 after discontinued suckling ($p<0.001$, $p<0.01$, respectively). Surprisingly, localization of claudin-1 signals was not limited to the apicolateral membrane of epithelial cells, but also to the basolateral membrane of the cells. Moreover, localization of claudin-4 was relocated from apicolateral membrane areas to the basolateral membrane, whereas total expression was not changed ($n=4$-7, respectively).

Conclusions: In mammary gland epithelium not only expression changes of single claudins were observed, but also a distinct relocation of these proteins. These results indicate that claudins may not only contribute to barrier function but also to adhesive functions ensuring structural integrity of mechanically challenged mammary gland epithelium during lactation. These mechanisms might have major effects on milk composition, and might be induced by pressure and milk ingredients, vice versa.

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Workshop: New aspects in physiology and nutrition of sows regarding actual developments in breeding

Workshop: Neue Aspekte der Physiologie und Ernährung von Sauen in Anbetracht der züchterischen Entwicklungen

WS1 Genetic factors on reproductive traits in pigs

Genetische Faktoren für Reproduktionsmerkmale beim Schwein

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In pig breeding litter size is an important characteristic of efficiency. Reproductive traits have low to moderate heritability and are modulated by many environmental and genetic effects. In fact, reproductive performance depends on genetic effect of the dams, but also of the sires and the offspring. Moreover, the dam serves as an environmental factor - in-uteri and post-partum - on the offspring, thus largely affecting the number of offspring born and weaned. Though the experimental design to obtain QTL for reproductive traits is complex, for example requiring an additional generation, linkage studies have been successfully performed for female functional and morphological traits related to conception, pregnancy, birth and rearing. Accordingly, the PigQTLd (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index) reports some 1200 QTL of 28 reproduction traits. SNP-chips are a tool that enable genome wide association studies in commercial herds and will therefore facilitate the detection of genes associated with reproductive traits. Global transcriptome analyses by microarray hybridization or next-generation-sequencing are used to address the molecular pathways relevant to fertility and early development. Especially the communication between maternal and embryonic/fetal tissues has been analyzed, as well as differences among high and low prolific breeds. Pig breeding have successfully selected for higher litter size also using DNA assisted selection. However, larger litters are partly associated with increased within-litter variation in birth weight, decrease in birth weight, greater pre-weaning mortality and reduced meat performance. Thus appropriate breeding measures are required to combat the current challenges resulting from great litters. Thus the mothering ability of the sow, which is related to the number of functional teats, plays an important role to warrant low piglet mortality. Teat number is a moderately heritable trait and a several QTL for number of functional and non-functional teats, i.e. inverted teats, were detected. Comparative expression studies of epithelial and mesenchymal teat tissue of normal and inverted teats at various developmental stages revealed differential expression of genes related to `cell maintenance, proliferation, differentiation and replacement´, `organismal, organ and tissue development´ and `cell-to-cell signaling and interaction´. The differential expression at fetal stages likely reflects the prenatal initiation of postnatal phenotypes concerning the number and shape as well as functionality of teats. In particular genes encoding members of canonical pathways of growth factor signaling were highlighted and associations with emergence of inverted teats were shown.

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Physiological aspects of lactation capacity in sows

Physiologische Besonderheiten der Sau in der Gravidität und Laktation

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The sufficient milk supply is one of the most relevant factors in suckling piglets during their first weeks of life. Beside an adequate supply of nutrients and electrolytes a variety of different essential components such as kolostral antibodies and regulatory factors for gastrointestinal development and differentiation is ensured by suckling. Thus, regulation of blood glucose and thermoregulation in newborn piglets are both directly associated with milk yield of the sow.

In recent years the number of piglets which are raised per year and sow has continuously been increased. Thus, it has to be questioned whether the lactational yield in sows has been increased respectively to meet the nutritional requirements in newborn piglets.

In contrast to ruminants a number of methodical difficulties exacerbate quantitative measurements of milk yield in sows which are related with both, anatomical and functional factors. Since cisterns are lacking in sows teats milk can only be collected after reflexive milk ejection with an average duration between 10 and 20 sec. In addition, suckling frequency can range around 20 per day which is difficult to simulate under experimental conditions.

Different methods have been established for quantitative measurements of milk production in sows. The weigh-suckle-weigh-method (WSW) is based on measuring differences in body weight before and after suckling which is an easy approach. However, defecation and urination as well as behavioral irritations may reduce the experimental accuracy of this method. An alternative approach is the isotope dilution technique as an invasive method by using deuterium oxide (D_2O) as a marker for measuring whole body water turnover. The major prerequisites are the availability of respective analytical devices and the lack of fluid intake except via milk during the experimental period. With a modified WSW technique body weight gain is recorded within a defined time interval.

The methodical difficulties are the major reason for the limited number of data on milk production. These data vary within a wide range between 4 and 10 kg/day depending on the breed and the lactational phase of the sow. In addition to daily milk volume the changes in milk composition as a function of time after parturition have to be taken into account. These changes are mainly reflected by the rapid decreases in immunoglobulin concentrations within approximately 48 hours after parturition which induce respective decreases in gross energy, crude protein concentrations and dry matter content in colostrum and milk. If the percentage of metabolizable energy in milk is assumed as 90% of gross energy a daily milk yield of about 10 kg would allow an adequate milk intake for 11 piglets which is lower than the average litter size. Thus, the additional supply of milk replacer has to be adjusted with regard to the ontogenetic development of intestinal functions in piglets within the first two to three weeks of life. These developmental processes are characterized by both, expression of the variety of enzymes and of respective transport proteins. Thus, feed supply to suckling piglets is associated with aspects of animal welfare when the discrepancy between reproductive performance and lactational yield is considered.

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Zur Energie- und Proteinversorgung der laktierenden Zuchtsau

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Es bedarf keines weiteren Hinweises, dass die Reproduktionsleistung von Sauen in den letzten zehn Jahren enorm zugenommen hat. In erfolgreichen Praxisbetrieben setzen heute Sauen durchschnittlich 12,5 Ferkel pro Wurf und über 30 Ferkel pro Jahr ab (Schnippe und Niggemeyer, 2014). Es ist zu erwarten, dass ein nicht unerheblicher Anteil der Tiere 13-15 Ferkel pro Wurf absetzt, dies bestätigen auch Beobachtungen an der Lehr- und Versuchsanstalt Futterkamp (Maier, pers. Mitt.). Ein sehr hoher Zuwachs des Wurfs wird jedoch nur erreicht, wenn Sauen eine Milchleistung von 14-15 kg pro Tag erbringen. Dies ist ein Leistungsniveau des 4,5-fachen Erhaltungsbedarfs und entspricht damit dem einer Kuh bei einer Milchleistung von 40 kg, wobei dieser Vergleich sicherlich etwas problematisch ist, da Sauen diese Leistung nur über einen relativ kurzen Zeitraum erbringen und ein wesentlich energiereicheres Futter erhalten.

Die Gesellschaft für Ernährungsphysiologie hat im Jahr 2006 Empfehlungen zur Versorgung mit Energie- und Nährstoffen von Zuchtsauen veröffentlicht (GfE, 2006). Die experimentellen Daten, die als Grundlage zu deren Ableitung dienten, sind jedoch wesentlich älter und wurden an Tieren mit geringeren Leistungen erhoben. Es ergibt sich zunächst die Notwendigkeit, die entsprechenden Tabellen für höhere Leistungen zu ergänzen, indem auch Versorgungsempfehlungen für sehr hohe Wurfzuwächse von 3,5 kg pro Tag angegeben werden. Die bisher gemachten Angaben für Wurfzuwächse bis 3,0 kg pro Tag decken jedoch auch den noch heute relevanten Leistungsbereich ab.

Die wissenschaftlich größere Herausforderung als die Erweiterung der bestehenden Tabellen besteht jedoch in der Frage, ob die bei deutlich niedrigeren Leistungen gemessenen Werte zum Erhaltungsumsatz und zur Effizienz der Verwertung von Energie und Aminosäuren auch für Tiere mit deutlich höherer Leistung zutreffend sind. Es ist daher die Frage zu prüfen, (1) ob der Erhaltungsumsatz bei Tieren mit hoher Milchleistung unverändert ist, und (2) ob eine unveränderte lineare Beziehung zwischen ME-Aufnahme und Energieabgabe über die Milch (inklusiv Energieansatz im Körper) besteht. Die bisher verwendeten Werte auch für höhere Leistungsbereiche zu unterstellen, wäre nur zulässig, wenn diese vom Leistungsniveau nicht beeinflusst werden; dann können Extrapolationen auf höhere Leistungen zu verlässlichen Schätzungen führen. Da beide Aspekte nicht durch Ergebnisse aus neueren Untersuchungen an Sauen selbst beantwortet werden können, soll versucht werden, ausgehend und unter Berücksichtigung der bisherigen Studien an Sauen, aufgrund von Untersuchungen an einer anderen Tierart (Milchkuh) sowie einer anderen Leistungsrichtung (Wachstum) dieser Frage nachzugehen.

In einer umfassenden Studie wurde bei Milchkühen geprüft, ob und inwieweit sich bei Tieren mit sehr hohen Leistungen Erhaltungsbedarf und Energieverwertung für die Milchbildung verändert haben (Agnew und Yan, 2000). Aus ihr ergab sich, dass in den Experimenten, die von den Arbeitsgruppen um Moe und Tyrrell in den USA bzw. um Van Es in den Niederlanden durchgeführt und in den 1970er Jahren publiziert wurden und Grundlage des in Deutschland eingeführten NEL-Systems sind, deutlich niedrigere Werte für den energetischen Erhaltungsumsatz (ME_m) und geringere Effizienzen für die Energieverwertung der Milchbildung (kl) bestimmt wurden als in späteren Experimenten der 1990er Jahre. Agnew und Yan (2000) gehen davon aus, dass die stark auf Milchleistung gezüchteten Rassen einen Bedarf für MEm im Bereich von 0,62 MJ ME/kg BW0,75 und einen kl-Wert von 0,66 aufweisen. Die entsprechenden Werte im aktuellen deutschen System (GfE, 2001) sind: ME_m = 0,49 MJ ME/kg $BW^{0,75}$ und kl = 0,60-0,62 für energiereiche Rationen. Die Linearität zwischen ME-Aufnahme und Energieabgabe über die Milch (inklusiv Energieansatz) und damit die Konstanz des k_l-Werts wurde bestätigt. Zu ganz ähnlichen Ergebnissen kommen Moraes et al. (2013). Diese in den vergangenen Jahrzehnten eingetretenen Veränderungen im Energiehaushalt führen jedoch bei einer Milchleistung von 30-35 kg nicht zu anderen Bedarfswerten, da sich der höhere MEm-Bedarf und die höhere Energieverwertung kompensieren. Der erhöhte MEm-Bedarf wird durch den deutlich gesteigerten Grundumsatz dieser Genotypen hervorgerufen. Dass heutige milchbetonte Rassen resorbierte Nährstoffe effizienter für die Synthese nutzen können, kann jedoch aus zwei Gründen mit hoher Sicherheit ausgeschlossen werden: (1) Wird der für Kauarbeit notwendige Energiebedarf als gesonderte Größe des Energieumsatzes berücksichtigt, ergibt sich auch aus den Ergebnissen der älteren Experimente ein k_l – Wert von ca. 0.64-0,66. (2) Die gute Übereinstimmung der Energieverwertung beim Wiederkäuer mit der energetischen Verwertung der aus dem Dickdarm resorbierten Nährstoffe beim Schwein (Susenbeth, 2005) zeigt, dass es sich hierbei um biochemische Stoffwechselvorgänge handelt, deren Effizienz kaum variiert, und daher auch keine bedeutsamen Veränderungen innerhalb einer Tierart zu erwarten sind.

Im Folgenden soll der sich daraus ergebenden Frage nachgegangen werden, ob ähnliche Veränderungen durch Leistungssteigerung wie bei der Milchkuh auch bei der laktierenden Zuchtsau eingetreten sein könnten. Zunächst kann festgestellt werden, dass keinerlei Hinweise auf solche Veränderungen in der Literatur zu finden sind. Auch in einem neueren Modell zur Versorgung und zum Bedarf von Sauen (Dourmad et al., 2008), das auf einer umfassenden Auswertung vorliegender Experimente beruht, wird unverändert von den auch bisher bekannten Werten ausgegangen: ME_m = 0,46 (0,44) MJ ME/kg BW0,75; kl = 0,72 (0,70); k_{rl} = 0,87 (0,89), wobei krl die energetische Verwertung der mobilisierten Energie in Milchenergie bedeutet und in Klammern die von der GfE (2006) verwendeten Werte angegeben sind.

Des Weiteren ist die Übersteinstimmung des k_l-Werts mit der Energieverwertung für das Wachstum (kpf) beim Schwein ins Auge fallend und auch bedeutsam. Denn für den kpf-Wert konnte festgestellt werden, dass (1) er unabhängig von der Höhe und der Zusammensetzung des Wachstums ist, (2) sich ein Wert ebenfalls von 0,70-0,72 ergibt, wenn die von Jentsch et al. (2000) publizierten nährstoffspezifische Verwertungen für übliche Rationen verwendet werden, und (3) Werte in der gleichen Größenordnung auch bei anderen monogastrischen Spezies auftreten. Daher scheint es begründet und nicht eine willkürliche Vereinfachung zu sein, eine hohe Konstanz und eine von der Leistungsrichtung unabhängige ME-Verwertung anzunehmen und diese auch für den kl-Wert zu unterstellen, zumal die Zusammensetzung der Milch nur geringen Schwankungen unterliegt.

Für die Effizienz der Protein- und Aminosäurenverwertung kann ebenfalls angenommen werden, dass die von der GfE (2006) verwendeten Werte unverändert auch für sehr hohe Leistungen Gültigkeit besitzen. Der Erhaltungsbedarf an Protein und Aminosäuren ist jedoch auf die Körpergröße bezogen; dieser dürfte daher aufgrund hoher Futteraufnahmen und bei den üblichen Gehalten an Faser im Futter deutlich höher liegen, wie auch neuere Studien am wachsenden Schwein nahelegen (Ringel und Susenbeth, 2009; Blank et al., 2012). Ein Bezug zur Futter- bzw. Faseraufnahme könnte daher erforderlich werden (wie dies auch bei der Standardisierung der praecaecalen Aminosäurenverdaulichkeit geschieht), da die endogenen Verluste im Darm zum überwiegenden Anteil hierdurch bedingt sind und den größten Anteil des Erhaltungsbedarfs ausmachen.

Die Ableitung des Protein- und Aminosäurenbedarfs aufgrund der Milchleistung ist mit hoher Genauigkeit möglich, da die Unsicherheiten beim Erhaltungsbedarf aufgrund ihres geringen Anteils kaum ins Gewicht fallen. Die praktische Umsetzung der Versorgungsempfehlungen bei der Rationsgestaltung oder Mischfutterherstellung durch Erreichen bestimmter Konzentrationen an Protein und Aminosäuren setzt jedoch die Kenntnis des Futteraufnahmevermögens voraus. Da die Futteraufnahme häufig nicht ausreicht, den Energiebedarf auch bei einer energiereichen Ration zu decken, kommt es zu dem bekannten Gewichtsverlust während der Laktation. Ein Gewichtsverlust bis zu 20 kg wird noch als unproblematisch angesehen, wenn die Tiere ausreichende Fettreserven aufweisen. Entsprechende Reserven an Körperprotein stehen jedoch nicht zur Verfügung, so dass eine Ration, die für eine bedarfsdeckende Situation konzipiert ist, auch den Bedarf an Protein und Aminosäuren nicht vollständig decken kann. Daher ist es erforderlich, deren Gehalt entsprechend zu erhöhen, um eine zu starke Körperproteinmobilisierung zu verhindern, die als Hauptursache für einen verzögerten Eintritt der Rausche angesehen wird.

Da die Tiere eines Bestandes nicht alle gleichermaßen von einer energetischen Unterversorgung, die durch die begrenzte Futteraufnahme und die Höhe der Leistung bestimmt wird, betroffen sind, sollten Fütterungsstrategien entwickelt werden, die gezielt eine Unterversorgung mit Protein und Aminosäuren (evt. auch mit Mineralstoffen und Vitaminen) verhindern. Dies könnte durch eine Ergänzung mit einem proteinreichen Futter oder durch proteinreichere Alleinfutter, die nur solchen Tieren angeboten werden, geschehen. So müsste beispielsweise der Proteingehalt von 19,5 % (mit 5,5 % Lysin im Futterprotein), der bei einer hohen Milchleistung und einer Futteraufnahme von 8 kg pro Tag den Bedarf deckt, bei einer geringeren Futteraufnahme von 7 kg pro Tag auf 22 % angehoben werden, um eine Proteinunterversorgung zu vermeiden. Eine pauschale Erhöhung des Gehalts für alle Tiere ist aus ökonomischen und umweltrelevanten Gründen nicht sinnvoll. Neben dem Bemühen um die Realisierung einer hohen Futteraufnahme sollte daher dem Aspekt der bedarfsdeckenden Protein- und Aminosäurenversorgung bei variabler Futteraufnahme eine größere Bedeutung beigemessen werden.

Zusammenfassend ist festzuhalten, dass die Grunddaten zur Ableitung der Empfehlungen zur Versorgung von Energie- und Nähstoffen (GfE, 2006) auch für Tiere mit hohen Leistungen als zutreffend anzusehen sind. Daher können die entsprechenden Tabellen auf dieser Basis erweitert werden. Bei der Rationsgestaltung ist darauf zu achten, dass unabhängig vom Grad der Energiebedarfsdeckung die notwendige Versorgung an

Protein und Aminosäuren sichergestellt wird.

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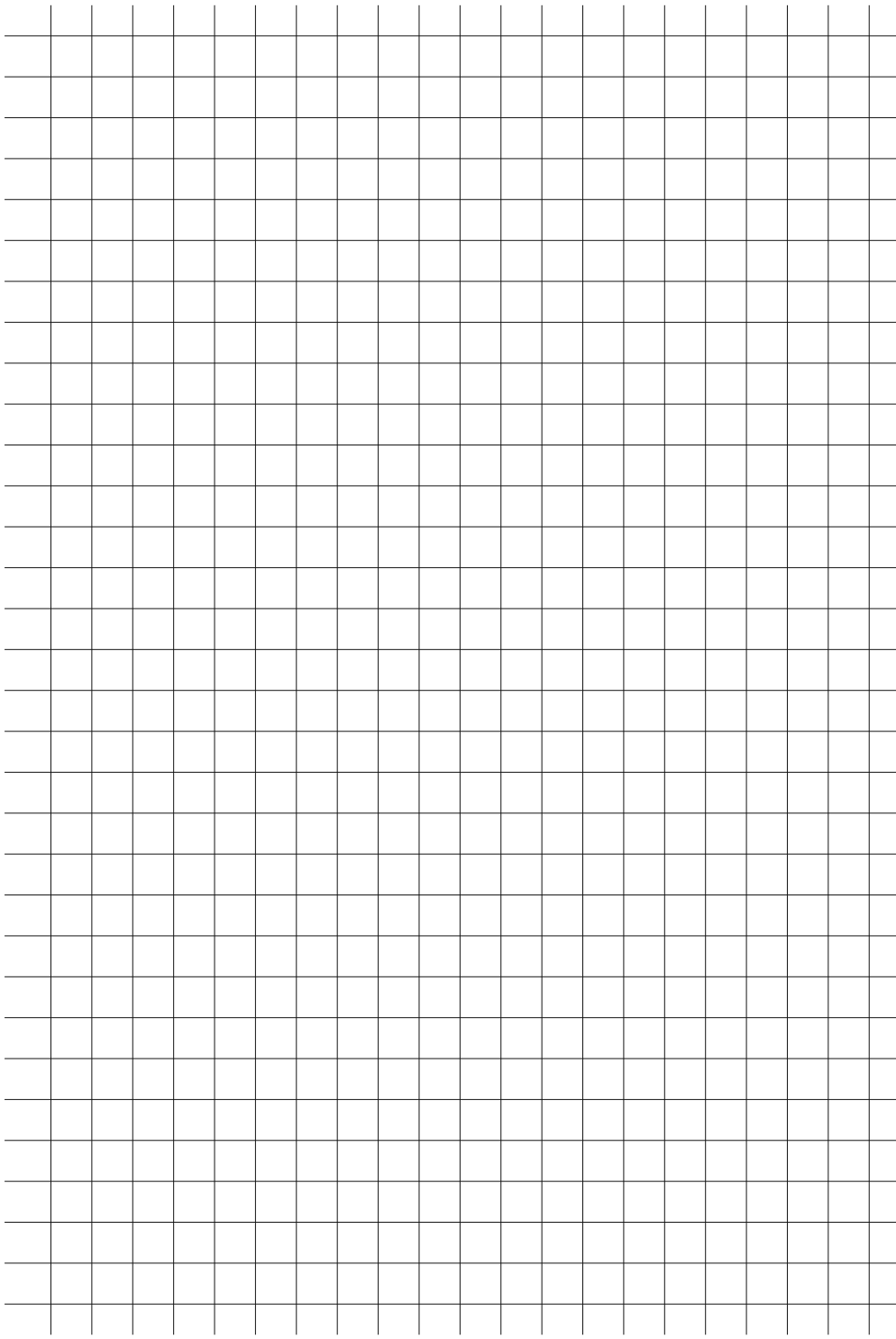

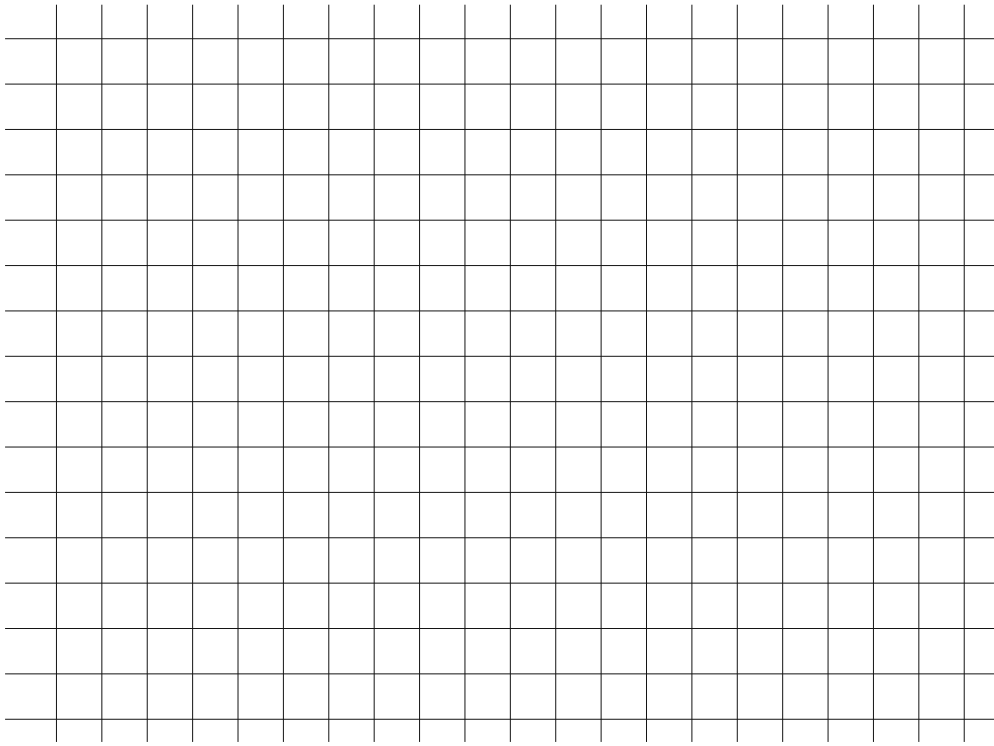

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