16.

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

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

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

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

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

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

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

*[1] HEMARAJATA, P. et al. (2013) Therap Adv Gastroenterol, 6:39-51*
*[2] BORDA-MOLINA. D. et al. (2016) Front Microbiol, 7: 2033*

*Institut für Nutztierwissenschaften, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany, daniele.borda@uni-hohenheim.de

17.

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

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

*Li Z., Sciascia Q. L., Görs S., Rayatdoost F., Chen W., Tuchscherer A., Metges C. C. – Dummerstorf/Guangzhou City

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

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

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

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

*(1) CAUGANT A., GUEBLEZ R. (1993) J Rech Porc de France, 24, 123-128.*
*(2) HALES J., ET AL. (2013) J Anim Sci. 91, 4991-5003.*
*(3) NISSEN P.M., ET AL. (2011) J Biomed Biotechnol. 10, 1-8.*

**Institute of Nutritional Physiology „Oskar Kellner“, Leibniz Institute for Farm Animal Biology (FBN), 18196 Dummerstorf, Germany; metges@fbn-dummerstorf.de.*

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

18.

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

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

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

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

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

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

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

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

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

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

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

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

19.

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

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

*Khan D. R., Siebert D., Agostini P. – Schwalbach am Taunus/Lelystad

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

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

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

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

[a-e] Figures with different superscripts are statistically significant ($P<0.05$)

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

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

*(1) Corzo, A. et al. (2007). J. Appl. Poult. Res. 16:546–554.*
*(2) Pastor e al. (2013). Poult. Sci. 92 :3184–3195*

*CJ Europe GmbH, Technical marketing, Ober der Roeth 4, 65824 Schwalbach am Taunus, Germany, dr.khan@cj.net

20.

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

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

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

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

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

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

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

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

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

*[1] DEAN, D.W., BIDNER, T.D. and SOUTHERN, L.L. (2006). Poult. Sci. 85:288-296*

*University of Hohenheim, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany, philipp_hofmann@uni-hohenheim.de