## **Nonruminant Nutrition: Enzymes and Minerals**

## **92** Supplemental dietary phytase alters gut microbiota of weanling pigs. L. Wang and X. G. Lei\*, *Cornell University, Ithaca, NY.*

Past phytase research has been largely focused on nutritional values of the enzyme, and has not explored its impact on gut microbiota of animals. The objective of this study was to determine effects of supplemental Escherichia coli AppA2 and Aspergillus niger PhyA phytases on composition changes of the 4 major intestinal bacteria in weanling pigs. A total of 30 crossbreds (3-week old, Yorkshire-Landrace-Hampshire) were allotted to 3 groups (n = 10) and fed a corn-soybean-mean basal diet (BD, supplemented with 0.35% inorganic phosphorus), the BD plus 3,500 units of AppA2/kg (Optiphos, JBS United, Sheridan, IN), or the BD plus 3,500 units of PhyA/kg (Natuphos, BASF, Florham Park, NJ) for 6 weeks. At the end, 8 pigs of each treatment group were killed to collect ileum and colon adherent samples for terminal restriction fragments length polymorphism analysis of 16s rRNA genes. After the total genomic DNA was extracted, the 16s rRNA genes were isolated using PCR with universal primer sets. Compared with the BD, both phytases increased (P < 0.05) Bifidobacteria, Clostridium, E. coli/ Salmonella, and lactobacilli in ileum. There was no consistent effect of either phytase on any of the detectable bacteria in colon except for that pigs fed AppA2 had slightly higher (P < 0.05) Bifidobacteria content than those fed PhyA. In conclusion, both AppA2 and PhyA phytases showed a stronger impact on microbiota in ileum than in colon. Our finding reveals a novel function of phytase beyond nutrition.

Key words: microbiota, phytase, swine, T-RFLP

**93** Effects of phytase on standardized total tract digestibility of P in copra expellers, palm kernel expellers, and palm kernel meal fed to growing pigs. B. L. Almaguer\*<sup>1</sup>, R. C. Sulabo<sup>2</sup>, and H. H. Stein<sup>2</sup>, <sup>1</sup>Universidad Autónoma de Querétaro, Mexico, <sup>2</sup>University of Illinois, Urbana.

A total of 66 barrows (initial BW:  $27 \pm 3$  kg) were used to determine the effects of phytase on standardized total tract digestibility (STTD) of P in copra expellers (CE). Asian palm kernel expellers from Indonesia (PKE-IN), African palm kernel expellers from Costa Rica (PKE-CR), African palm kernel meal from Costa Rica (PKM), and in soybean meal (SBM). Pigs were housed individually in metabolism cages and allotted to a randomized complete block design with 11 diets and 6 replicate pigs per diet. Five diets were formulated by mixing cornstarch and sucrose with CE, PKE-IN, PKE-CR, PKM, or SBM. Five additional diets identical to the initial 5 diets with the exception that they contained 500 units of phytase (OptiPhos 2000, Enzyvia, Sheridan, IN) were formulated. A P-free diet was used to measure basal endogenous P losses (EPL). Feces were collected for 5 d based on the marker to marker approach after a 5-d adaptation period. Analyzed total P in CE, PKE-IN, PKE-CR, PKM, and SBM was 0.52, 0.51, 0.53, 0.54, and 0.67%, respectively. Phytate P was calculated to be 0.22, 0.35, 0.38, 0.32, and 0.44% in CE, PKE-IN, PKE-CR, PKM, and SBM, respectively. Addition of phytase increased (P < 0.05; SEM = 5.0) the ATTD of P from 60.6 to 80.8, 39.1 to 56.5, 38.2 to 59.9, 48.9 to 64.1, and 48.7 to 73.5% in CE, PKE-IN, PKE-CR, PKM, and SBM, respectively. The basal EPL was estimated to be  $216 \pm 70$  mg/ kg DMI. The STTD of P increased (P < 0.05; SEM = 5.7) from 70.6 to 90.3, 49.4 to 66.4, 48.7 to 69.9. 57.9 to 73.5, and 57.3 to 81.1% in CE, PKE-IN, PKE-CR, PKM, and SBM, respectively, with added phytase. In summary, added phytase increased P digestibility of all the

test ingredients, and CE had greater STTD of P than PKE, PKM, and SBM when fed to growing pigs.

Key words: alternative feedstuffs, phosphorus, pigs

## **94** Supplementing a xylanase alone or a combination of xylanase and $\beta$ -glucanase on growth performance, health, and nutrient digestibility of nursery pigs. Y. Han\* and A. Ludger, *Nutreco R & D*, *Boxmeer, the Netherlands.*

It remains questionable if non-starch polysaccharide degrading enzymes should be used in pig diets. Two experiments were conducted to investigate the impact of adding a xylanase alone or a combination of xylanase and  $\beta$ -glucanase (XG) on nursery pigs fed a wheat-based diet. In Exp 1, 200 weaned piglets (28 d old, 7.1kg BW) were allocated to 10 blocks of 4 pens (5/pen). The 4 dietary treatments included a control diet and control with Xylanase (4000 U/kg), XG1 (xylanase 1500 U/kg, β-glucanase 200 U/kg) or XG2 (xylanase 2500 U/kg,  $\beta$ -glucanase 200 U/kg). All diets were pelleted and fed for 5 wks in a 2-phase program (d7-21, d22-42). In Exp 2, 32 piglets (28 d old, 8.5kg BW) were housed individually in metabolic cages. The same diets as in Exp 1 were used with 3% SiO<sub>2</sub> included as the marker for digestibility. A similar feeding program was followed. Fecal samples were collected in each phase and the ileal content was collected at the end of the study. In Exp 1, pigs fed the XG2 diet showed significant improvement on the cumulative feed efficiency in comparison to the control and the Xylanase diet. Compared with the control group, adding XG2 improved feed efficiency by 3.8% (P < 0.035). The Xylanase and XG2 treatment also reduced diarrhea incidence compared with the control (P < 0.049). In Exp 2, the treatment did not impact ileal digestibility. However, fecal digestibility differed significantly. Both XG1 and XG2 treatment improved ash digestibility (P < 0.042). Organic matter (P< 0.004) and NDF digestibility (P < 0.001) were improved by all 3 enzymes. While Xylanase and XG1 improved ADF digestibility ( $P \leq$ 0.002), energy digestibility was significantly enhanced by Xylanase and XG2. Besides, XG2 addition tended to increase protein digestibility (P = 0.08). In conclusion, Xylanase alone increased nutrient digestibility but failed to improve growth performance. A combination of xylanase and β-glucanase XG2 improved nutrient digestibility, feed efficiency and health of the animal.

Key words: piglets, xylanase, β-glucanase

**95** Effect of different dietary calcium concentrations on the digestive and metabolic response of growing pigs to microbial phytase. X. Rousseau<sup>\*1,2</sup>, M. P. Letourneau-Montminy<sup>3</sup>, M. Magnin<sup>2</sup>, A. Narcy<sup>1</sup>, and C. Pomar<sup>3</sup>, <sup>1</sup>INRA UR<sup>83</sup> Poultry Research, Nouzilly, France, <sup>2</sup>BNA Animal Nutrition, Chateau-Gontier, France, <sup>3</sup>Agriculture and Agrifood, Lennoxville, QC, Canada.

An experiment was conducted to assess the effect of dietary calcium (Ca) concentration on the response of growing pigs to microbial phytase in terms of phosphorus (P) and Ca utilization at the digestive and metabolic levels. Sixty 3 pigs, for each period, were fed a 2-phase feeding experiment (25–50 and 50–80 kg BW) according to a  $3 \times 3$ factorial arrangement in which dietary Ca (5.8, 7.2, 8.4 g/kg for phase 1 and 4.1, 5.8, 7.4 g/kg for phase 2) and microbial phytase (0, 350 and 700 FTU/kg for both phases) were provided at constant P concentrations (4.8 g/kg and 3.9 for phases 1 and 2, respectively). Pigs

were individually fed while raised in one large group. At the beginning and at the end of each period pigs were weighed and 4 pigs per treatments scanned with Dual energy x-ray absorptiometry (DXA) to estimate total body and lumbar spine region (L2-L4) bone mineral content (BMC) and density (BMD). After slaughter, femur was removed and scanned with DXA. No differences were observed among treatments for ADG and ADFI during the first feeding period, whereas during the second period ADG was depressed by dietary Ca concentration (P =0.003) while ADG and ADFI were both increased by phytase (respectively, P = 0.036, P = 0.026). Total body and L2-L4 BMD (respectively, P < 0.001, P = 0.002) and BMC (P < 0.001, P < 0.05) were linearly increased by microbial phytase. Similarly, femur BMD and BMC were linearly increased by microbial phytase for first (respectively, P = 0.021; P < 0.001) and second (respectively, P < 0.001; P < 0.001) feeding periods. Regardless of the feeding phase, dietary Ca concentration had no effect on bone mineralization. This last criterion was improved by microbial phytase independently of Ca.

Key words: calcium, phytase, pigs

**96** Effects of supplemented NSP-degrading enzymes on nutrient digestibility of diets containing wheat and wheat millrun fed to grower pigs. D. Shrestha<sup>\*1</sup>, J. Broz<sup>2</sup>, and R. T. Zijlstra<sup>1</sup>, <sup>1</sup>University of Alberta, Edmonton, AB, Canada, <sup>2</sup>DSM Nutritional Products, Animal Nutrition and Health R&D, Basel, Switzerland.

A critical issue of current swine production is high feed cost that might be ameliorated by co-products including wheat millrun. However, feedstuffs such as millrun have physicochemical limitations such as a high non starch polysaccharide (NSP) content. The NSP hinder nutrient digestibility but are also a potential energy source if hydrolyzed by bacteria or NSP-degrading enzymes. The objective was to determine the effect of NSP-degrading enzymes on diets containing wheat or wheat and wheat millrun on nutrient digestibility. Effects of diet (96% wheat or 56% wheat plus 40% wheat millrun) and xylanase (0 or 16,000 units xylanase and 15,600 units  $\beta$ -glucanase/kg of feed) were investigated in a  $2 \times 2$  factorial arrangement with a N-free control diet for a total of 5 diets. Five pigs were fed 5 diets in a  $5 \times 5$ Latin square. Arabinoxylans constituted 50 and 57% of the total NSP in wheat and millrun, respectively. The wheat used in this study contained 3.80 Mcal DE/kg of DM, 16% CP, and 11% NSP, whereas the millrun contained 2.90 Mcal DE/kg of DM, 17% CP, and 25% NSP. Supplementation of NSP-degrading enzymes to the wheat diet did not alter the apparent ileal digestibility (AID) of energy, AID of CP, or the apparent total tract digestibility (ATTD) of energy. Supplementation of NSP-degrading enzymes to the wheat millrun diet increased (P <0.05) the AID of energy and CP by 5% and increased (P < 0.05) the ATTD of energy by 5%. The improved energy digestibility of the millrun diet was supported by an increase (P < 0.05) of the AID and ATTD of NSP by 36 and 47%, respectively. Supplementation of NSP-degrading enzymes increased (P < 0.05) the content of ileal digested energy and DE of millrun by 0.34 and 0.41 Mcal/kg of DM. In conclusion, exogenous NSP-degrading enzymes match with the NSP contained in wheat millrun and can improve energy and protein digestibility of diets containing wheat millrun for grower pigs.

Key words: pig, wheat millrun, xylanase

97 Capillary electrophoresis coupled with inductively coupled plasma mass spectrometry (CE-ICP-MS) enables identification and quantification of copper and manganese glycinate complexes in enriched feed samples and the study of their bioavailability. C. Ionescu\*<sup>1</sup>, V. Vacchina<sup>2</sup>, R. Lobinski<sup>3</sup>, and D. Bravo<sup>1</sup>, <sup>1</sup>Pancosma, Geneva, Switzerland, <sup>2</sup>UT<sup>2</sup>A, Pau, France, <sup>3</sup>CNRS, Pau, France.

Copper and Manganese glycinate complexes (BT Cu and BT Mn) are introduced in feeds, but up to date, there was no analytical method enabling their identification and quantification in such matrixes. The first objective was to check if capillary electrophoresis coupled with inductively coupled plasma mass spectrometry (CE-ICP-MS) could be used to do so.

Key words: traceability, glycinate complexes, feed

**98** Effects of feeding tribasic copper chloride or copper sulfate on growth and efficiency of nursery pigs. E. A. Koutsos<sup>\*1</sup>, G. L. Allee<sup>2</sup>, and T. J. Prince<sup>3</sup>, <sup>1</sup>Micronutrients, Indianapolis, IN, <sup>2</sup>PorkTech LLC, Columbia, MO, <sup>3</sup>Prince Nutrition Service LLC, Auburn, AL.

Tribasic copper chloride (TBCC) is a covalently bonded copper source that has been shown to have significantly higher bioavailability in monogastrics and ruminants than copper sulfate (CuSO<sub>4</sub>). The objective of this trial was to determine the effects of adding graded levels of copper (Cu) from TBCC on performance of nursery pigs and compare TBCC to 200 ppm Cu from CuSO<sub>4</sub>. 1188 pigs weaned at 20 +/- 2 d of age were allotted by weight and sex into 48 pens (8 reps of 6 treatments, 24-25 pigs/pen). Treatments were added Cu levels from TBCC at 15, 61.25, 107.5, 153.75, or 200 ppm and 200 ppm Cu from CuSO<sub>4</sub>. Pigs were fed complex diets for phase 1 (0-7 d) and phase 2 (7-21 d) and a corn-soy diet for phase 3 (21-42 d). Added Zn levels (from ZnO) were 3000 ppm in phase 1 and 2500 ppm in phase 2 and all diets contained an AGP. Increasing dietary Cu from TBCC linearly increased BW at d 7 (P < 0.04), d 21 (P < 0.01) and d 42 (P < 0.01). Addition of Cu from TBCC increased ADG for 0-7 d (P < 0.02), 0–21 d (P < 0.01) and 0–42 d (P < 01). G:F improved linearly (P < 0.01) 01) with increasing Cu from TBCC for 0-42 d. Feeding 200 ppm Cu from TBCC increased BW (P < 0.06), ADG (P < 0.02), and improved G:F (P < 0.12) for phase 1 compared with 200 ppm Cu from CuSO<sub>4</sub>. Break-point analysis showed the optimum level of Cu from TBCC to be 150 ppm for ADG at d 0–21, 143 ppm for ADG at 0–42 d, and 133 ppm for the 0-42 d G:F. Cu from TBCC is effective for improving ADG and G:F of nursery pigs.

Table 1.

						Significance		
Cu Source	TBCC					CuSO <sub>4</sub>	(P <)	
Cu level,								Cu
ppm	15	61.25	107.5	153.75	200	200	Linear	source
Init. Wt.,								
kg	5.28	5.27	5.27	5.28	5.28	5.27		
D21 Wt.,								
kg	11.44	11.51	11.80	11.87	12.02	11.94	0.01	0.70
Final Wt.,								
kg	22.46	23.06	23.75	24.05	24.44	23.97	0.01	0.29
D0-7								
ADG,								
kg/d	0.13	0.13	0.14	0.14	0.16	0.13	0.02	0.02
G/F	0.81	0.76	0.80	0.84	0.77	0.68	0.22	0.12
D0-21								
ADG,								
kg/d	0.29	0.29	0.31	0.31	0.32	0.32	0.02	0.01
G/F	0.75	0.75	0.75	0.76	0.76	0.77	0.20	0.54
D0-42								
ADG,								
kg/d	0.41	0.42	0.44	0.45	0.45	0.44	0.02	0.01
G/F	0.66	0.67	0.67	0.68	0.68	0.68	0.01	0.88

Key words: copper, nursery pig, tribasic copper chloride

**99** Intestinal, liver, kidney, serum and biliary Cu concentrations in piglets fed Cu proteinate or CuSO<sub>4</sub>. B. Aldridge<sup>\*1</sup>, R. F. Power<sup>2</sup>, K. A. Dawson<sup>2</sup>, and S. Radcliffe<sup>1</sup>, <sup>1</sup>Purdue University, Department of Animal Science, West Lafayette, IN, <sup>2</sup>Center for Animal Nutrigenomics and Applied Animal Nutrition, Alltech, Nicholasville, KY.

Eighty crossbred barrows were weaned at  $20 \pm 1$  d of age and used in 2 blocks (5 reps/block) of a  $2 \times 3$  factorial experiment to investigate the effects of Cu source (CuSO<sub>4</sub> and Bioplex Cu, Alltech Inc.) and concentration (4, 25, and 125 ppm Cu) on growth performance, serum ceruloplasmin activity and Cu concentration in proximal jejunum (PJ), liver, kidney, serum and gall bladder contents (GBC). A negative control diet containing no supplemental Cu was also fed. Pigs were blocked by BW and randomly assigned to diets offered in 2 daily feedings at 9% of metabolic BW for 14 d. The PROC MIXED procedure in SAS was used to determine the main and interactive effects of Cu source and concentration. In addition, PROC GLM linear and quadratic contrasts were determined for increasing Cu concentrations. Pig served as the experimental unit. Overall ADG and G:F did not differ between Cu sources (P > 0.05). However, ADG and G:F approached a positive linear trend (P = 0.12 and P = 0.11, respectively) as dietary Cu was increased from 0 to 125 ppm Cu. During wk2, ADG and G:F (227.8, 227.9, 267.1 and 260.7 g/d, P = 0.07; and 0.59, 0.56, 0.68 and 0.65, P = 0.06, respectively) tended to increase in a quadratic fashion for pigs fed 0, 4, 25, or 125 ppm from Bioplex Cu. Serum ceruloplasmin activity was not altered (P > 0.05) by Cu source or concentration

fed. An interaction (P < 0.02) between Cu source and concentration was observed for serum Cu concentrations. When dietary Cu was fed at 125 ppm, serum [Cu] was 12% higher for Bioplex Cu fed pigs compared with CuSO<sub>4</sub> fed pigs was observed in the serum, compared with CuSO<sub>4</sub>, though when fed. However, at 25 ppm, serum [Cu] was 10% lower for Bioplex Cu fed pigs. There were no (P > 0.10) interactive effects of Cu source and concentration, nor were there any effects of Cu source observed for Cu concentrations in PJ, liver, kidney and gall bladder. However, increasing dietary Cu linearly increased (P < 0.001) Cu concentration in the PJ, liver, kidney, serum and gall bladder contents. These data suggest that Cu from CuSO<sub>4</sub> and Bioplex Cu accumulate in a positive linear fashion in the PJ, liver, kidney and GBC, but can differ in the serum.

Key words: copper, pig, absorption

**100** Effect of dietary calcium on gastric ulceration in yearling horses. C. W. Waters<sup>\*1</sup>, D. H. Sigler<sup>1</sup>, N. D. Cohen<sup>2</sup>, and P. G. Gibbs<sup>1</sup>, <sup>1</sup>Texas A&M University Department of Animal Science, College Station, <sup>2</sup>Texas A&M University College of Veterinary Medicine, College Station.

Equine gastric ulcer syndrome (EGUS) is a complex and common disorder observed in horses used in many different events, from part-time show horses to performance and race horses. Previous studies have indicated alfalfa hay to be beneficial in reducing severity of EGUS. Due to possible buffering effect of significantly higher concentration of calcium in alfalfa hay compared with Bermuda grass hay, understanding the role of calcium in gastric ulceration is needed. The objective of this study was to evaluate the effect of high Ca diet on severity of EGUS in young horses. Nineteen yearling Quarter Horses were used in a randomized cross over study, and were fed either a high Ca (1.85%) diet or a normal (1.03% Ca) pelleted diet for 28-d periods, separated by a 21-d washout period. At the beginning and end of both periods, horses were evaluated endoscopically and EGUS scores were assigned by a veterinary practitioner using a 1-4 scoring system. Initial EGUS score averaged 1.4 at the beginning of the trial and were not different between treatment groups. Horses tended to increase (more severe) in EGUS score while confined in dry lots and individually fed either control or high Ca diet for 28-d. Diet had no effect on EGUS (P = 0.334), or on change in EGUS score (P = 0.42). The sequence in which diets were fed tended to effect EGUS score (P = 0.095) with a mean ulcer score of 2.3 for horses fed the treatment diet followed by control vs. 1.9 for horses fed control followed by treatment. Horses that received the control diet initially had a longer period to adjust to the lower Ca diet before being put on the high Ca diet. Reported causes of EGUS are multiple and are interrelated to stress and diet. In this study, there appeared to be little effect of added Ca on severity of EGUS. Other nutrient differences between alfalfa and coastal Bermuda grass hay warrant further investigation.

Key words: equine gastric ulcer syndrome, EGUS