Nonruminant Nutrition: Enzymes 1

331 Efficacy of a thermally processed exogenous enzyme cocktail on broiler performance. K. R. Beaman*, K. G. S. Lilly, L. K. Shires, S. A. Loop, and J. S. Moritz, *West Virginia University*, *Morgantown*.

Feed ingredient price has influenced nutritionists to maximize diet nutrient availability through use of exogenous enzymes. Poultry are almost exclusively fed pelleted diets that entails feed being subjected to conditions of high moisture, temperature and pressure that could partially denature added enzymes. Exogenous enzyme efficacy may be decreased or completely lost if enzymes are not able to survive the pelleting process. The objective of the current studies was to properly assess the efficacy of a commercially available exogenous enzyme cocktail subjected to increasing steam conditioning temperatures during pelleting (82, 88, 93oC). All studies used male Cobb 500 broilers obtained from the same commercial hatchery. The experimental period was from d 3–21 with 8 birds per pen and 8 replications per treatment. Study 1 established significant differences between the Positive Control (PC) and Negative Control (NC) diets ($P < 0.05$). However, the exogenous enzyme cocktail did not show improved performance. Study 2 was designed to improve the opportunity for the exogenous enzyme cocktail to demonstrate a benefit. This study utilized increased mixer-added lipid addition in the diet formulation that may decrease frictional heat production in the pellet die, and a decreased metabolizable energy difference between the positive and negative control. Again, performance differences were observed between the PC and NC control diets ($P < 0.05$), with no beneficial effect demonstrated for the exogenous enzyme at any temperature ($P > 0.05$). In Study 3, diet formulations were similar to Study 2; however, temperatures were decreased (71, 77, 82oC) and an additional unconditioned mash (UCM) treatment was added. Significant differences were obtained between NC diets with and without exogenous enzyme cocktail in the UCM for live weight gain and feed conversion ratio (FCR) and at 82oC for FCR ($P < 0.05$). However, only numerical differences in performance were shown between the PC and NC diets.

Key Words: exogenous enzyme cocktail, enzyme efficacy, broiler performance

332 Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multi-carbohydrase enzyme. T. A. Woyengo*[1], B. A. Slominski[1], and R. O. Jones[2], [1]*Department of Animal Science, University of Manitoba*, *Winnipeg, Canada*, [2]*Canadian Bio-Systems Inc.*, *Calgary, Canada*.

An experiment was conducted to determine the effect of supplementing a corn-soybean meal-based diet with phytase alone or in combination with citric acid (CA) or multi-carbohydrase, a preparation of non-starch polysaccharide-degrading enzymes (MC) or both on growth performance and nutrient utilization. A total of 360 one-day-old broiler chicks were assigned to 6 dietary treatments, each consisting of 12 pens of 5 birds each, and were fed experimental diets from 1 to 21 d of age. The diets included a positive control (PC) (0.46% non-phytate P; 1.1% Ca), a negative control (NC) (0.26% non-phytate P; 0.89% Ca), and NC without or with phytase (600 U/kg) alone, phytase plus CA (5 g/kg), phytase plus MC (Superzyme OM; 0.6 g/kg), or phytase plus CA and MC. Birds fed the PC diet had higher ($P < 0.05$) BWG (764 vs. 594 g/21 d) and tibia ash content (50 vs. 38%) than those fed the NC diet. Phytase improved ($P = 0.03$) BWG (632 g/21 d), which increased further ($P = 0.018$) to 673 g/21 d for the phytase plus MC diet. In contrast to phytase alone, phytase plus MC supplementation improved ($P < 0.05$) FCR of the NC diet from 1.37 to 1.32. Tibia ash content for the NC diet increased ($P < 0.05$) from 38 to 42% due to phytase and a trend ($P = 0.136$) in its further increase to 44% was noted for the phytase plus MC diet. Phytase improved ($P < 0.05$) ileal digestibility of P from 29 to 43%, and the addition of CA or MC or both to phytase-supplemented diet further increased ($P < 0.05$) P digestibility to 52, 53 and 54%, respectively. Phytase addition improved ($P < 0.05$) diet AMEn content from 2959 to 3068 kcal/kg, which tended ($P < 0.06$) to increase further following CA (3150 kcal/kg) or MC (3142 kcal/kg) addition. No interactions were detected between CA and MC on all response criteria measured. The results show that addition of MC to the phytase-supplemented broiler diets can result in improved nutrient utilization and growth performance.

Key Words: broiler, enzyme, citric acid

333 Intestinal histology and amino acid digestibility of broilers fed increasing dietary phytic acid during a live coccidia vaccination. R. N. Lehman*[1], A. J. Cowieson[2], C. L. Walk[1], and A. P. McElroy[1], [1]*Virginia Tech*, *Blacksburg,* [2]*AB Vista*, *Wiltshire, Marlborough, UK*.

Day-old Cobb 700 male broilers were obtained from a commercial hatchery, weighed, and half were spray-vaccinated with a live coccidia vaccine (Coccivac-B) before placement into one of 72 floor pens with clean pine shavings (35 chicks/pen). Vaccinated and non-vaccinated birds were given one of 3 diets with different phytic acid (PA) levels (low = 0.20% phytate-P; medium = 0.28% phytate-P; high = 0.36% phytate-P) for a total of 6 vaccination X diet treatments (12 replications/treatment). The non-vaccinated birds received no coccidiosis control. On d 21, ileal digesta was collected for amino acid (AA) digestibility and duodenal, jejunal, and ileal tissue samples were collected for histological examination. In the duodenum, there was a significant diet by vaccination interaction ($P < 0.05$) on villus height (VH) with non-vaccinated birds having a shorter VH than vaccinated birds on the low PA diet, but a greater VH than the vaccinated birds when fed the medium and high PA diets. The VH to crypt depth (CD) ratio (VCR) remained constant for the non-vaccinated birds as dietary PA increased, whereas VCR decreased ($P < 0.05$) in the vaccinated birds as PA increased. Vaccinated birds and birds given the medium PA diet had deeper ($P < 0.05$) crypts compared with all other treatments. In the jejunum, vaccinated birds had deeper ($P < 0.05$) crypts and larger VCR. Vaccinated birds fed the low PA diet had larger VH than non-vaccinated birds, but a smaller VH than non-vaccinated birds fed the medium and high PA diets. In the ileum, vaccination alone caused a larger ($P < 0.05$) VH, CD, and VCR. Vaccination caused a decrease in total AA digestibility ($P < 0.05$), and the high PA diet resulted in the highest AA digestibility. These results suggest that interactions between the level of dietary PA and coccidia vaccination could alter intestinal morphology and subsequently have an effect on AA digestibility in broilers. An improved AA digestibility seen with the high PA diet may be due to exceeding a critical PA:protein ratio that may cause protein insolubility at intermediate levels.

Key Words: phytate, vaccination, histology

334 Effects of NSP-enzymes on in vitro digestibility and intestinal microbiota activity in broilers fed two different wheat cultivars. B. Bouza, C. Clavaud, P. A. Geraert, and E. Devillard*, *ADISSEO SAS*, *03600 Commentry, France*.

Non-starch polysaccharide (NSP) enzymes or carbohydrolases are commonly used in poultry diets to improve feed digestibility. These enzymes modify substrates reaching the different parts of the gastrointestinal tract and thus could have similar effects to that of prebiotics on intestinal microflora. The aims of the present study were to determine in vitro the effects of carbohydrolase complex (Rovabio Excel) on the digestibility of 2 wheat cultivars, Caphorn (Ca) and Isengrain (Is) differing by their NSP composition, and on the consequences on broiler intestinal microbiota activities. A first in vitro incubation step was carried out to mimic digestion in the upper digestive tract with pepsin/HCl and pancreatin incubations with supplementation or not with carbohydrolases (Rovabio Excel AP at 0.5 mg/g substrate). In vitro digestibilities of energy (dE) of the 2 cultivars were different, with Ca being less digestible than Is (−3%, $P < 0.009$). The effects of Rovabio depended on wheat cultivars, with an improvement of dE of 6.4% ($P = 0.005$) and 2.1% ($P = 0.081$) for Ca and Is, respectively. The resulting materials were used in an anaerobic incubation with ileal contents from broilers. More gas (+38 mL, $P = 0.0004$) and more short chain fatty acids (SCFA) (+164 m*M*, $P = 0.025$) were produced with Ca than Is in relation with dE of the wheat cultivars (Is > Ca). When carbohydrolases had been used in the first incubation step, there was a decrease in gas and SCFA productions. These effects were more important for Ca than for Is. Finally, the enzymes effects were also observed on SCFA profile, with more butyrate and less propionate produced from enzyme-treated substrates than from untreated substrates. In conclusion, this study showed a positive effect of NSP-enzymes on the in vitro digestibility of energy of both wheat cultivars, which leads to changes in intestinal microbiota characteristics. There were also differences between cultivars, in term of effects of NSP-enzymes, probably linked to NSP composition of the two substrates.

Key Words: NSP-enzymes, wheat digestibility, ileal fermentation

335 Assessment of phytase in broilers undergoing a coccidiosis challenge. A. L. Shaw*, J. P. Blake, and K. S. Macklin, *Auburn University*, *Auburn, AL*.

An experiment was conducted to assess the effects of a phytase enzyme on broilers undergoing a coccidiosis challenge through 21 d of age. Twenty one days before the experiment, 120 chicks were placed in 24 of 48 floor pens to produce a coccidia challenge. At 10 d of age they were orally dosed with a cocktail containing 100,000 and 5,000 sporulated *E. acervulina* and *E. tenella* oocysts, respectively, for litter seeding. Straight run broiler chicks (1008) were placed across 48 floor pens (21 birds/pen, 6 reps/trt) on either fresh or seeded litter. All birds were fed a corn-soybean meal diet (22% CP, 3087 kcal/kg) adequate in all nutrients but Ca and available phosphorus (aP). Treatments were created using a combination of 2 Ca-aP levels (0.9% Ca, 0.45% aP vs. 0.7% Ca, 0.25% aP and 500 FTU Optiphos), 2 coccidia challenges (unchallenged vs. challenged), and 2 vaccination strategies (unvaccinated vs. vaccinated with CoccivacB prior to placement). On d 10, 18, and 21 bodyweight (BW) and feed consumption (FC) were recorded for each pen. Five birds/trt were sacrificed and intestinal samples were obtained for visual and microscopic lesion scoring on d 10 and 18. At 21 d 18 birds/trt were selected for removal of left tibia to assess bone strength. BW and FC were unaffected (P>0.05) by inclusion of phytase or vaccination strategy. From 0-10d birds exposed to the seeded litter had a higher FCR (P<0.05). Upon conclusion of the experiment, birds exposed to coccidia had lower BW and FC, as well as a higher feed conversion (P<0.05) in comparison to those not challenged. Regardless of treatment, bone breaking force as well as visual and microscopic scoring of the duodenum and ceca showed no differences (P>0.05). Although there were no statistical differences in cocci scoring, incidence of cocci was greater in challenged vs. unchallenged birds. Results indicate that phytase was ineffective in improving the performance or P utilization of birds vaccinated and/or subjected to a coccidiosis infection.

Key Words: Broiler, *Eimeria* spp., phytase

336 Dietary supplementation of *Peniophora lycii* phytase improves mineral bioavailability in broiler chickens. A. Kollanoor Johny*[1], K. Syam-Mohan[1], T. V. Viswanathan[1], and A. Jalaludeen[2], [1]*Department of Animal Nutrition, College of Veterinary and Animal Sciences, Kerala Agricultural University*, *Mannuthy, Kerala, India*, [2]*Centre for Advanced Studies in Poultry Science, College of Veterinary and Animal Sciences, Kerala Agricultural University*, *Mannuthy, Kerala, India*.

An investigation was carried out to study the effect of dietary *Peniophora lycii* phytase on the growth and mineral bioavailability in broiler chickens. Day-old, straight run commercial broiler chicks (n = 96) were randomly divided into 8 identical groups containing 12 birds each, reared under deep litter system for 8 weeks. The groups were randomly allotted to 2, maize-soy based diets: control and experimental, with 4 replicates per treatment. Birds in the control group received a standard broiler ration (SBR) whereas, the treatment group received SBR supplemented with phytase at 750 U/kg diet. Feed and water were supplied *ad libitum.* Body weight and feed intake were recorded, and feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated. Two birds from each replicate were sacrificed at sixth and eighth week and, liver, spleen, tibia and blood samples were collected for analyses. At the end of 8 weeks, a 3-d metabolism trial was carried out using 2 birds selected randomly from each replicate, and housed in individual metabolism cages with facilities for feeding, watering and collection of droppings. Body weight, dry matter (DM) intake, FCR and PER did not differ significantly between the groups ($P > 0.05$). Also, DM and nitrogen retention between the groups were not different. However, the availability of calcium (Ca), phosphorus (P), magnesium (Mg), copper (Cu), and iron (Fe) was significantly improved ($P < 0.05$) with phytase supplementation and were 39, 55, 54, 24, and 65% more, respectively compared with the control. The concentration of Fe in the liver was significantly higher ($P < 0.05$) in the phytase-treated groups at sixth and eighth week of trial, whereas its concentration in the spleen did not differ between groups. Tibial weight and tibial ash content at sixth week were significantly higher ($P < 0.05$) for the phytase-treated groups. However, serum concentrations of Ca, P, Mg, Cu, Fe and Zn did not differ between the groups. Results of the study indicate that *Peniophora lycii* phytase could potentially improve the bioavailability of minerals in broiler chicken.

Key Words: *Peniophora lycii* phytase, mineral bioavailability, broiler chicken

337 Mineral excretion and bone mineral content as affected by phytase and feed additives in broilers. M. R. Dalmagro*[1], E. O. Oviedo-Rondón[1], A. Mitchell[2], A. B. Leytem[3], N. A. Barbosa[4], N. K. Sakomura[4], J. W. Wilson[5], and C. Paulus[5], [1]*North Carolina State University*, *Raleigh,* [2]*USDA-ARS, BARC*, *Beltsville, MD*, [3]*USDA-ARS*, *Kimberly, ID*, [4]*Universidade Estadual Paulista, UNESP*, *Jaboticabal, SP, Brasil*, [5]*DSM Nutritional Products Inc.*, *Parsippany, NJ*.

One broiler study was conducted to evaluate the effects of feed additives (FA) in diets containing phytase on mineral excretion and bone mineral content (BMC) for broilers up to 43 d of age. Corn-soybean diets with 5% inclusion of DDGS were used as basal diets. All diets contained ionophore Coban. The treatments evaluated consist of a control without phytase (C), and 7 treatments that contained phytase Ronozyme P

formulated to release 0.1% of phytate phosphorus (P) to have an equal amount of available P compared with C. These 7 treatments included diets without any other FA (PC), GP antibiotic (BMD), 3 probiotics: BC30 (*B. coagulans*), B2B (*B. licheniformis*, *B. subtilis*), and Calsporin (*B. subtilis*), and 2 EO: Crina POULTRY Plus (CPP) at 300 ppm and Crina PoultryAF (CPF) at 100 ppm. Day-old Ross 708 broilers were randomly assigned to 96 floor pens with previously used litter. Ten males and 10 females were placed in each pen. Fresh excreta were collected at 40 d and one male per pen was sacrificed at 44 d of age to collect bones. Data were analyzed as a completely randomized block design with 12 replicates per treatment. The highest excretion of P (Pex) and water extractable P (WEP) was observed in C chickens. Excreta from C chickens had the highest moisture, but the lowest K and Mg. These chickens also had the lowest tibia and femur BMC. The Pex was reduced by addition of phytase in all diets. GP and the EO did not cause further reductions on Pex, but all probiotics reduced Pex. WEP was reduced by PC, GP and CPP. Excreta of chickens fed diets containing only phytase had the highest concentration of Ca, Mn, and Mg. Calcium excretion was reduced by probiotics and EO. The highest tibia and femur BMC was observed in B2B chickens. The excreta of these B2B chickens had the highest concentration of K and the lowest concentrations of Na, Zn, and Mn. Concentrations of Cu in the excreta were not affected by treatments. It was concluded that FA may affect mineral metabolism in chickens fed diets with phytase. Probiotics seem to have a positive effect on P retention and bone mineral deposition.

Key Words: probiotics, essential oils, growth promotants

338 Use of the precision-fed rooster assay and a chick AME trial to determine the best method for enzyme efficacy. J. Brandon* and A. B. Batal, *The University of Georgia*, *Athens*.

The beneficial effects of exogenous enzyme addition to poultry diets have been well documented. However, the results have been variable depending on the in vivo method used. The most common methods employed to assess the bioefficacy of exogenous enzymes in vivo are performance trials and metabolism studies, such as the precision-fed rooster assay. There has been criticism of the use of the precision-fed rooster assay. Thus, the objective of these studies was to compare the precision-fed rooster assay with the chick AME trial to determine which method best measures enzyme efficacy. In 2 studies 6 different enzymes mixed in a complete diet (as well as a control diet with no enzyme, for a total of 7 treatments per study) were evaluated using the precision-fed rooster TME assay alongside a more conventional chick AME trial. The rooster assays were traditional precision-fed rooster assays in which 8 birds per diet were fasted for 24 h then crop intubated with 35 g of the test diet and excreta was then collected for 48 h. However, to keep consistent with the chick AME trial, the roosters were 'primed' on the experimental diets. They were allowed ad libitum access to the experimental diets for 5 d before the fasting and crop intubation. The chick AME trials used 728 d old Cobb 500 by-product male chicks (7 replications per treatment, 8 chicks per replication) that were fed the same experimental diets for 5 d and excreta was collected at 18 d of age for the determination of AME. Regardless of the method used, in both studies no significant increase was observed due to exogenous enzyme supplementation on the TME values determined from the precision-fed rooster assays or the AME values determined from the chick digestibility trials. The determined TME values were significantly higher than the AME values. However, no correlation was observed between the determined ME values for the control or enzyme diets between the rooster and the chick assay, suggesting the assay used could affect the measured efficacy of the enzymes.

Key Words: enzymes, TME, AME

339 The effects of the addition of phytase and an enzyme cocktail to high and low nutrient density diets with DDGS or MBM in laying hens during phase II. D. Hahn*[1], S. Scheideler[1], E. E. M. Pierson[2], and C. L. Novak[3], [1]*University of Nebraska-Lincoln*, *Lincoln,* [2]*Danisco Animal Nutrition*, *St. Louis, MO,* [3]*Land O' Lakes Purina Feed, LLC*, *Kansas City, MO, and Lincoln, NE*.

The objective was to test the addition of Avizyme 1502, a blend of protease, amylase and xylanase (Danisco, UK Ltd.) in laying hen diets containing dried distillers grains (DDGS) or meat and bone meal (MBM) during phase II of egg production. All diets contained phytase (300 FTU minimum; Phyzyme XP 5000 G Feed Enzyme). Three hundred eighty-four Hy-Line W-36 laying hens were used in this study, from 35 to 52 weeks of age. There were 12 replicate pens with 4 hens per pen. The experiment consisted of 8 dietary treatments arranged in a 2 × 2 × 2 factorial design: diets (DDGS or MBM), metabolizable energy (ME) levels (2880 Kcal/kg or 2800 Kcal/kg), and 2 enzyme levels (0 or 0.0375% Avizyme) to provide protease at 8000U/g, amylase at 800U/g and xylanase at 600 U/g of product. All diets contained Phyzyme at 60 g MT (~300 FTU) and were formulated to contain 0.30% avP and a Ca adjustment as recommended by Phycheck software tool (10% decrease). Response variables measured included: daily egg prod, biweekly egg wt, weekly feed intake; body wt, Haugh unit, yolk wt, albumen wt, shell wt, shell strength and specific gravity were taken monthly. There was a significant effect for feed intake between high and low ME diets ($P = 0.0349$), with diet 3 (2880 Kcal/kg, MBM, Phyzyme) having significantly lower intake when compared with the other 7 treatments. However, there were no differences noted between treatments for hen weight, egg production and egg quality parameters ($P > 0.05$). Thus, reducing ME, P and Ca with the addition of enzymes had no negative effect on egg production and quality. Given Nov 2009 commodity prices, there was a cost savings shown in regards to high (2880 Kcal/g) vs low (2800 Kcal/g) energy diets when MBM was in the diet. There was a cost savings with the addition of Avizyme.

Table 1. Kg feed cost/Doz. eggs produced

	DDGS		MBM	
ME	-Avzym	+Avzym	-Avzym	+Avzym
2880	$0.253	$0.227	$0.322	$0.240
2800	$0.251	$0.238	$0.269	$0.261

Key Words: metabolizable energy, Avizyme1502, laying hens

340 Justifying phytogenic feed additive matrix values in conjunction with exogenous feed enzymes. L. K. Shires*, S. A. Loop, C. K. Gehring, K. R. Beaman, and J. S. Moritz, *West Virginia University*, *Morgantown*.

Phytogenic feed additives (PFA) are purported to possess antimicrobial properties as well as nutrient sparing characteristics that may aid in alleviating high diet costs; however, in order for PFAs to assist nutritionists in decreasing diet cost, matrix values must be determined and implemented in feed formulation. On d4, 1,344 male Cobb 500 broilers were weighed and randomly allotted to 1 of 64 floor pens. Floor pens were located in 2 separate rooms, composed of one block each. Study 1 evaluated proposed matrix values for a commercially available PFA and assessed nutrient sparing when the product was combined with commercial phytase, carbohydrase and protease. The most remarkable proposed matrix values were 14.6 kcal/lb for metabolizable energy and 0.07% for Ca and AP. The objective of Study 2 was to determine true amino acid digestibility (TAAD) and nitrogen corrected true metabolizable energy (TME_n) using 32 cecectomized SCWL roosters. Dietary

treatments for both studies included a basal, basal with phytogenic product matrix value, basal with phytogenic product matrix value and phytogenic product, and similar treatments evaluating the phytogenic product matrix with exogenous enzyme products. Decreasing the basal diet by the proposed phytogenic matrix values decreased broiler live weight gain and increased feed conversion ratio ($P \leq 0.05$). However, when the same diet included the phytogenic feed additive, live weight gain and feed conversion ratio were restored to that of the basal diet ($P > 0.05$). The proposed matrix values of the specific PFA tested were justified; however, the PFA was not additive or synergistic with exogenous enzymes. Nitrogen corrected true metabolizable energy and TAAD data did not differ when the diets varied based on the PFA per se ($P > 0.05$). However, when the PFA was incorporated using proposed matrix values and used in conjunction with exogenous enzyme matrix values, several tested TAAD values were decreased ($P \leq 0.05$). Decreased nutrient digestibility may involve reductions in gut microflora due to the PFA as well as simultaneous reduction in substrate concentrations.

Key Words: phytogenic feed additives, matrix values, true amino acid digestibility

341 The effect of phytase and energy enzyme inclusion on growth and bone ash in low phosphorus diets. J. R. Coppedge*[1], J. Klein[1], K. Jessen[1], A. Jordan[1], B. Brown[2], F. Ruch[2], and J. T. Lee[1], [1]*Texas A&M University*, *College Station,* [2]*Enzyvia LLC*, *Sheridan, IN*.

An experiment was conducted to evaluate the effect of varying levels of phytase with and without NSPase inclusion on broiler performance when supplemented in corn/soybean meal diets low in available phosphorus. The objective was to determine if NSPase inclusion enhances phytase activity in relation to growth parameters and bone ash in broilers reared in batteries through 14 d of age. Four diets with selected available phosphorus levels of 0.15%, 0.20%, 0.25%, and 0.30% were included in the experimental design to develop a dose response curve to calculate phosphorus release from experimental treatments. An additional 6 treatments were evaluated that included 3 levels of phytase (150, 200, and 250 FTU/kg) with and without NSPase inclusion in a diet containing 0.15% available phosphorus. Evaluated parameters included body weight, feed conversion ratio, mortality and bone ash percentage. Body weight and bone ash percentage were positively influence with increases in available phosphorus levels. Phytase inclusion positively influenced growth performance and bone ash percentage. Broilers fed the 200 and 250 FTU/kg phytase inclusion levels outperformed the broilers fed the 150 FTU/kg inclusion level. Addition of NSPase with 150 FTU/kg phytase resulted in increased broiler body weight as compared with the 150 FTU/kg phytase diet alone. Using regression equations determined from dose response treatments for body weight, bone ash (mg), and bone ash percentage, NSPase inclusion increased phosphorus release at the 150 FTU/kg level from 0.06% to 0.09%. These data indicate that NSPase inclusion may increase phytase effectiveness when co-administered during early stages of growth.

Key Words: bone ash, phytase, broiler