**544** Utilizing solanum glaucophyllum and phytase to improve phosphorus utilization in broilers . Y-H Cheng<sup>\*1</sup>, J.P. Goff<sup>2</sup>, J.L. Sell<sup>3</sup>, S Gill<sup>4</sup>, E. Pawlak<sup>4</sup>, M. Elena<sup>4</sup>, and R.L. Horst<sup>2</sup>, <sup>1</sup> lowa State University/Biomedical Science, <sup>2</sup> National Animal Disease Center, <sup>3</sup> lowa State University/Animal Science, <sup>4</sup> CAE, Buenos Aires, Argentina.

Both 1,25-dihidroxyvitamin D3 and phytase are known to improve phosphorus utilization in broilers(Biehl and Baker, J Nutr 1997). Solanum glaucophyllum(Sg) is a plant containing high amounts of 1,25dihydroxyvitamin D bound to a glycoside. It may be an economical source of 1,25-dihydroxyvitamin D for animal feeds. We examined the effect of Sg, with and without phytase, on phosphorus utilization in broilers fed corn-soybean meal diets. The basal diet was 0.6% Ca, 0.45% total P(0.15% available P) with adequate vitamin  $\mathrm{D3}(3000\mathrm{IU/kg}).$  The six treatments included basal diet, basal with 7.5g Sg/kg, basal with 10 g Sg/kg, basal with 1200<br/>FTU phytase/kg,<br/>basal with phytase (1200FTU/kg) and Sg (7.5g/kg), and "normal" diet (0.7% total phosphorus, 1% calcium). Each treatment was given to six pens of 8 chicks. When compared with the basal diet, addition of 7.5 gSg/kg or phytase improved growth and bone mineralization. Increasing Sg to 10 g/kg diet did not significantly improve weight gain or bone density over the 7.5 g Sg/kg dose. Phytase and Sg supplementation increased plasma and bone Ca and P concentration similarly, however the greatest weight gain and tibiae ash was observed in the phytase (1200FTU/kg) group. Adding 7.5 g Sg to the phytase supplementation did not significantly improve P utilization over phytase alone.

Diet	Blood P	Bone ash	Bone density	Weight gain
	mg/dl	g/tibiae	$\rm g/cm^3$	gw
Basal(B)	4.56a	1.022a	1.117a	984a
B+7.5gSg	6.89b	1.194c	1.132b	1045b
B+10gSg	7.02b	1.246c	1.128ab	1046b
B+phytase	7.06b	1.348d	1.136b	1136c
B+phytase+7.5gSg	7.46b	1.375d	1.137b	1124c
Normal	7.54b	1.488b	1.158c	1033a

Key Words: Solanum Glaucophyllum, Phytase, Phosphorus

545 Effect of wheat bran phytase subjected to different conditioning temperatures on phosphorus utilization by broiler chicks based on body weight and toe ash measurements. W. B. Cavalcanti<sup>\*</sup>, K. C. Behnke, R. S. Beyer, and M. Okot-Kotber, *Kansas State University, Manhattan, KS*.

Cereal grains and oilseed byproducts are among the major components of diets fed to poultry. Although phosphorus levels are high in these ingredients, most of it is presented in phytate form and thus, unavailable. Wheat bran is known for having high phytase enzyme activity. A study was conducted with the objective of evaluating the efficacy of wheat bran in improving utilization of phytate phosphorus by broiler chicks. Four diets with increasing calculated levels of available phosphorus (Pay) (0.35, 0.40, 0.45, 0.50%) were used as references. Four test diets were formulated to contain 0.35% Pav. Three diets had a 5% inclusion of wheat bran subjected to different treatments: unconditioned, conditioned at  $60^{\circ}$ C and  $80^{\circ}$ C. The fourth diet had no wheat bran, but a commercial source of phytase. All treatments were isocaloric, isonitrogenous and formulated to meet or exceed NRC recommendations. Diets in the mash form were used in a (0-3) weeks battery study. Regression equations were used to estimate levels of Pav in the test diets. Increasing levels of Pav in reference diets resulted in improved live weight gain (LWG) and percent toe ash (PTA) on a dry matter basis. When utilized in the unconditioned form, wheat bran provided similar results for LWG and PTA to those obtained with the commercial phytase diet. Although some additional phosphorus was estimated to be available in those diets, due to phytase activity, the levels of the mineral were still below those recommended by the NRC. Increasing conditioning temperature  $(60^{\circ}C \text{ and } 80^{\circ}C)$  lowered LWG and PTA of birds fed those diets. The results lead to the conclusion that wheat bran can be utilized in low Pav diets for broilers without any negative effects in LWG and PTA measurements when compared with diets containing proper levels of phosphorus. It may also be suggested that wheat bran cannot be conditioned or pelleted without losing most of its enzyme activity.

Key Words: Wheat bran, Phytase, Broiler

**546** Evaluation of the available phosphorus requirement & optimal phytase level of **21-42** day old male broilers. J. R. Timmons<sup>\*1</sup>, J. M. Harter-Dennis<sup>1</sup>, and A. E. Sefton<sup>2</sup>, <sup>1</sup>University of Maryland Eastern Shore, Princess Anne, MD, <sup>2</sup>Alltech, Inc., Guelph, Ontario, Canada.

Two trials were conducted to determine the available phosphorus (AP) requirement of 21-42 day old male broilers fed a corn & soybean meal diet. Also, the optimal dose & the phosphorus-sparing effect (PSE) of added phytase (Allzyme<sup>®</sup>, Alltech, Inc.) was evaluated. A RCB design was used in both trials. Weight gain (WG), feed efficiency (G/F), tibia breaking strength (TBS) & % tibia ash (TA) were used as dependent variables. Trial 1 was conducted to determine the AP requirement of 21-42 day old male broilers. Treatments included 5 levels of AP (0.15, 0.20. 0.25, 0.30, & 0.35%). WG results were 829.1 <sup>b</sup>, 910.7<sup>ab</sup>, 886.4<sup>ab</sup> 949.1<sup>ab</sup> & 1026.7<sup>a</sup>, respectively. There was no effect (P>0.05) of treatments on G/F. TBS & TA values were 24.2 <sup>d</sup> , 33.3 <sup>c</sup> , 35.7 <sup>bc</sup> , 38.9<sup>ab</sup> , 40.5<sup>a</sup>kg, & 45.5<sup>c</sup>, 47.9<sup>b</sup>, 51.4<sup>a</sup>, 53.5<sup>a</sup>, 53.5<sup>a</sup>% for diets containing 0.15, 0.20, 0.25, 0.30 & 0.35% AP, respectively. Using TA the AP requirement was determined to be 0.29%. Trial 2 was designed to confirm the AP requirement from trial 1 & determine the optimum phytase level & PSE of added phytase. Treatments included 4 levels of AP (0.15, 0.20, 0.25, & 0.30%) & 4 levels of phytase (2875, 5750, 8625, & 11500 PTU/kg). All phytase diets contained 0.15% AP. WG & G/F values were 925.8 <sup>b</sup> 947.7 <sup>b</sup>, 1011.8<sup>ab</sup>, 1198.1<sup>a</sup>g, & 0.283 <sup>b</sup>, 0.338<sup>ab</sup>, 0.322<sup>ab</sup>, 0.421<sup>a</sup> in birds fed 0.15, 0.20, 0.25, & 0.30% AP, respectively. There were no differences (P>0.05) in WG & G/F between any of the phytase treatments. Both TBS & TA increased as the level of AP or phytase increased in the diet. TBS results were 13.2 <sup>e</sup>, 18.2 <sup>bc</sup>, 20.2 <sup>b</sup>, 23.5<sup>a</sup>, 14.7 <sup>de</sup>, 17.0 <sup>cd</sup>, 18.3  $^{bc},$  & 18.0  $^{bc}\mathrm{kg}$  (AP & phytase levels, respectively). Using TA the AP requirement was determined to be 0.22%. The phytase level that maximized TA was determined to be 6769 PTU/kg. Also, the PSE of added phytase was estimated to be 0.04%. These results suggest that the AP level recommended by NRC may be high for 21-42 day old broilers.

Key Words: Phytase, Available phosphorus, Allzyme

**547** Reduction in dietary phosphorus concentration does not change brush border phytase activity along the small intestinal axis in broiler chicks. E. M. Onyango<sup>\*1</sup>, E. K. Asem<sup>2</sup>, and O. Adeola<sup>1</sup>, <sup>1</sup>Department of Animal Sciences, <sup>2</sup>Department of Basic Medical Sciences, Purdue University.

A study was conducted to determine if intestinal brush border phytase activity in chicks could be altered by decreasing the phosphorus (P) content in the diet. A deficiency in P could lead to upregulation of enzyme systems involved in the digestion of its compounds. Two groups, each of six 7-d-old male broiler chicks, were fed either a P-adequate diet or a low-P diet for 2 wk. The corn-soybean meal based diet contained  $10.1~{\rm g}$  Ca and 7.4 g total P or 10.6 g Ca and 3.4 g total P per kg for P-adequate or low-P diet, respectively. The low-P diet had no inorganic P added. The broiler chicks were killed on day 21, the small intestine was removed, cleaned and divided into the duodenum, jejunum and ileum. Mucosa was scrapped and used to prepare intestinal brush border vesicles by the magnesium chloride precipitation method. Phytase activity (nanomoles Pi/mg protein/min) in the vesicles was measured at pH 6.0 using sodium phytate as substrate. Released Pi was reacted with ammonium molybdate to form a blue complex and quantified spectrophotometrically at 620 nm. Phytase activity (Mean SEM) in chicks fed P-adequate diet was 36.695 8.163, 21.087 4.486 and 4.706 0.125 in the duodenum, jejunum and ileum, respectively. Activity in chicks fed low-P diet was 29.732 2.610, 18.821 3.672 and 8.781 0.613 in the duodenum, jejunum and ileum, respectively. There was no significant difference in phytase activity in the duodenum, jejunum and ileum of chicks fed P-adequate diet and those fed low-P diet. Phytase activity was highest in the duodenum followed by the jejunum and lowest in the ileum in chicks fed either diet. It is concluded that, at the level of P used, P content in the chick diet does not seem to affect brush border phytase activity. Regardless of dietary P concentration, there was a proximal to distal decrease in phytase activity along the small intestinal

 ${\sf Key}$  Words: Broiler chick, Phytase, Phosphorus, Brush border membrane vesicle

#### **548** Power of two methods for the estimation of bone ash of broilers. L. E. Hall<sup>\*</sup>, R. B. Shirley, R. I. Bakalli, S. E. Aggrey, G. M. Pesti, and H. M. Edwards, Jr., *University of Georgia*.

An experiment was conducted to compare two common methods of estimating bone ash from growing broiler chicks (A = autoclaving; B = boiling/extracting). Ross x Ross day-old-broiler chicks were fed a cornsoy, phosphorus deficient diet (22.7% CP; 1% Ca; 0.2219% AP), with either 0, 750, 1500, 3000, or 6000 units of phytase. Method A was compared to method B at 7, 14 and 21 days of age by sacrificing chicks from each treatment and removing one leg from each bird for analysis by each method. Using method A, bones were autoclaved, cleaned of flesh and cartilage ends, oven dried (105  $^{\circ}\mathrm{C}$  for 24 hr), weighed and ashed at 600  $^{\circ}\mathrm{C}$ overnight, allowed to cool, and weighed. Using method B, bones were boiled, cooled, cleaned of flesh (not cartilage), extracted for 24 h with ethanol and 24 h with anhydrous ether, dried, and weighed and ashed as above. At d-7, bone ash ranged from  $33.5\pm0.59$  to  $46.1\pm0.14\%$  for method A, and  $25.8\pm0.58$  to  $38.2\pm0.24\%$  for method B. At d-14, bone ash ranged from  $39.6\pm0.06$  to  $49.0\pm0.9\%$  for method A, and  $33.3\pm0.7$ to 42.60.3% for method B. At d-21, bone ash ranged from  $39.6\pm0.6$  to  $49.0\pm0.9\%$  for method A, and  $33.3\pm0.7$  to  $42.6\pm0.3\%$  for method B. To detect a 2% difference in bone ash for any two treatments at a 5 and 90% power, it would require a sample size (n) of 52 and 51 for methods A and B, respectively at d-7; an n of 63 and 44 for methods A and B, respectively at d-14; and an n of 114 and 40 for methods A and B, respectively at d-21. The variability in method A increased with age, while the reverse was observed using method B. In conclusion, both methods can be used to determine bone ash, however, more numbers are required for the autoclaving method compared to the boiling/extracting method, at the same power of estimation.

Key Words: Broiler chicks, Phytase, Bone ash

# **549** Effect of dietary iron overload on plasma total antioxidant capacity and hepatic lipid peroxides in chickens. Jennifer Cosgrove\*, Denzil Maurice, and Stephen Lightsey, *Clemson University, Clemson, SC 29634.*

We previously reported that dietary iron overload increased ascorbic acid (AA) synthesis and hepatic lipid peroxides and decreased storage of AA. These experiments involved sacrificing the animal; thus, it was desirable to find a method to estimate hepatic iron concentration (HIC) and oxidative/antioxidant status without killing the animal. Therefore, this experiment examined a possible correlation between HIC and plasma total antioxidant capacity (PTAC). Plasma antioxidant capacity can be easily measured from fresh plasma. Forty-eight 1-day-old Ross x Arbor Acre were separated into 4 groups of 16 birds and placed on one of 4 diets. The diets were a corn-soy starter diet containing a basal concentration of 250 mg/kg Fe, supplemental iron was provided as ground FeSO<sub>4</sub>·7H<sub>2</sub>O. Diet analysis showed the control diet contained an average of 250 mg/kg Fe, and diets 2, 3 and 4 contained 1233, 5123, and  $10633~\mathrm{mg/kg}$  respectively. The birds were maintained on these diets for 4 weeks before blood, kidney, and liver were collected. Response variables measured included: PTAC, plasma and liver AA, AA synthesis, hepatic and renal Fe, Cu, & Zn, and hepatic lipid and protein oxidation. The mortality rate was 50% in the birds fed diets 3 and 4. Those that survived exhibited a 500% depression in body weight and 500% increase in HIC compared to treatment 1 & 2. As expected, there was a significant decrease in stored AA (p<0.001) and increase in hepatic lipid peroxide values (p<0.01) in the highly overloaded animals. Results of the measurement of PTAC showed a similar trend in that levels from treatments 3 & 4 were significantly higher than levels from treatments 1 & 2 (p < 0.001). These results show that measuring PTAC by this method can be useful when there is severe iron overload. From the pattern of iron overload seen in these birds, we cannot say with certainty that PTAC is effective or ineffective in detecting smaller differences in HIC.

**Key Words:** Hepatic Iron, Plasma Antioxidant Capacity, Ascorbic Acid Metabolism

**550** Cloning and sequence analysis of manganesecontaining superoxide dismutase(MnSOD) cDNA in chickens. X. G. Luo<sup>\*1</sup>, Y. Q. Bu<sup>1</sup>, S. F. Li<sup>1</sup>, C. Lu<sup>2</sup>, Y. W. Li<sup>2</sup>, T. D. Crenshaw<sup>3</sup>, X. Kuang<sup>1</sup>, B. Liu<sup>1</sup>, J. F. Li<sup>1</sup>, and S. X. Yu<sup>1</sup>, <sup>1</sup>Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, P. R. China, <sup>2</sup>Southwest Agricultural University, Chongqing, P. R. China, <sup>3</sup>University of Wisconsin, Madison, U. S. A..

The objective of this study was to identify the entire nucleotide sequence of MnSOD cDNA in chickens. Manganese is of particular importance for chickens. Previous studies by our laboratory demonstrate that MnSOD activity was significantly higher in heart than liver and other tissues for both broilers and laying hens. Only heart MnSOD was affected by dietary Mn levels in practical corn-soybean meal basal diets. The entire sequence of MnSOD cDNA in these chickens has not been cloned and defined. The sequence would aid future investigations of heart MnSOD gene expression as affected by dietary Mn. Therefore, in this study, a 990bp cDNA fragment encoding MnSOD from broiler chicken heart was amplified by 3'RACE (Rapid Amplification of cDNA Ends) with degenerate primers designed on the N-terminal amino acid sequence of chicken MnSOD. The amplified fragment was cloned and sequenced. The 5'terminal region of the MnSOD transcript was also determined by using 5'RACE procedure. The 5'RACE fragment is 521bp in length, including 352bp of the overlapped region with the 3'RACE fragment. The MnSOD cDNA in chicken heart is 1108bp in length, including 25bp of the 5'untranslated region, 675bp of the coding region, and 408bp of the 3'untranslated region. The coding region encodes a peptide of 224 amino acid residues, in which there is a signal peptide of 26 amino acids and a mature peptide of 198 amino acids. The molecular weight of the mature MnSOD protein is 22KD. Comparisons of the deduced amino acid sequence with those of human, rat, C. elegans, and D. melanogaster show the amino acid homology of 82.4%, 84.7%, 62.4%, and 59.3%, respectively. Supported by the National Foundation (Premier Foundation) of Outstanding Young Scientists of China in NSFC (Project No: 39925028).

Key Words: Cloning and sequence analysis, MnSOD cDNA, Chickens

### 551 Comparative bioefficacy of Natuphos phytase versus peniophora lysii phytase. M.B. Coelho, B.W. Cousins\*, J Braun, and W.F. McKnight, *BASF Corporation, New Jersey*.

Two hundred fifty two day old broilers were utilized in a 3X3 factorial design (10 replications/treatment) to determine the bioefficacy of Natuphos <sup>®</sup> phytase compared with peniophora lysii phytase using a monocalcium phosphate standard. A phosphorus deficient corn/soybean diet was used as the control. This phosphorus deficient diet was supplemented with monocalcium phosphate ( 0.2, 0.4 and 0.6 g P/kg) or phytase (100, 200 and 300 U/kg). Bioefficacy was compared based on weight gain regression analysis. Regression equations of phytase effect on weight gain were y=0.3247x + 761,  $R^2$ = 0.8122 and y =0.147x + 761, R<sup>2</sup>=0.0658 for Natuphos and peniophora lysii phytase, respectively. Weight gain increased by 0.325 and 0.147 g per phytase unit for Natuphos and peniophora lysii phytase. Natuphos required fewer (p<.05) phytase units than peniophora lysii to reach 902 g bw (434 and 959 U, respectively). The bioefficacy of Natuphos and peniophora lysii phytase based on weight gain regression analysis were 100% and 45%, respectively.

Key Words: Phytase, Bioefficacy, Broilers

**552** Phosphorus sparing effect of phytase, **25**hydroxycholecalciferol, and citric acid when fed to broiler chicks. R. Angel<sup>\*1</sup>, A. S. Dhandu<sup>1</sup>, T. J. Applegate<sup>2</sup>, and M. Christman<sup>1</sup>, <sup>1</sup>University of Maryland, <sup>2</sup>Purdue University.

An experiment was done to determine the non-phytate phosphorus (nPP) sparing effect of three feed additives (phytase (PHY), 25hydroxycholecalciferol (25OHD3) and citric acid (CA)). Day-old, male Ross 308 chicks were placed in floor pens and fed a starter diet that met all NRC (1994) recommendations until 14 d of age. On d 14, 120 battery pens (eight birds per pen, eight pens per treatment) were assigned to fifteen treatments. The experiment was designed as a complete factorial arrangement of three PHY (0, 200 and 500 FTU/ kg), two 25OHD3 (0 and 70  $\mu$ g/kg) and two CA (0 and 3%) levels added to a low nPP (0.16%) and 0.80% calcium diet. Three additional diets that contained graded levels of nPP (0.24, 0.32 and 0.40%) were used to determine a regression curve against which improvements in P utilization, based on tibia ash, were measured. Performance measures were determined from 14 to 24 d of age. All birds were sampled at 24 d and the right tibia removed for drv-defatted ash determination. Body weight gain was not affected (P>0.05) by any of the feed additives but feed consumption was lower (P < 0.05) when CA was added. Feed-to-gain was positively affected (P<0.05) by the addition of PHY and CA. There was a main effect (P < 0.05) of PHY, 25OHD3 and CA on tibia ash. No interactions were seen on any of the variables measured. When tibia ash was regressed on diet nPP a linear regression was obtained (35.01 + 41.19\*nPP,  $r^2=0.83$ , P<0.05). Using this equation for tibia ash, the nPP sparing effect for the different feed additives was calculated. All three feed additives had a positive impact (P<0.05) on sparing effect but no interactions were seen. The sparing effects of the feed additives alone were 0.014, 0.048, 0.035 and 0.030% nPP when 200, 500 FTU PHY/kg, 3% CA, 70 µg 25OHD3/kg, respectively were added to the diet. The sparing effect when the highest levels of all three feed additives were used together was 0.116% nPP.

Key Words: Broilers, Citric acid, Phytase, 25-hydroxycholecalciferol, Non-phytate phosphorus

**553** Non-phytate phosphorus sparing effect of phytase and citric acid when fed to poults. R. Angel\*<sup>1</sup>, T. J. Applegate<sup>2</sup>, M. Christman<sup>1</sup>, and A. S. Dhandu<sup>1</sup>, <sup>1</sup>University of Marlyand, College Park, <sup>2</sup>Purdue University.

An experiment was done to determine the non-phytate phosphorus (nPP) sparing effect due to phytase (PHY) and citric acid (CA). Dayold, male Nicholas poults were placed in floor pens and fed a starter diet that met all NRC (1994) recommendations until 8 d of age. On d 8, 120 battery pens (nine birds per pen, eight pens per treatment) were assigned to fifteen treatments. The experiment was designed as a complete factorial arrangement of three PHY (0, 300 and 600 FTU/kg) and four CA (0, 1, 2 and 3%) levels added to a low nPP (0.44%) diet containing 1.20% calcium. Three additional diets were added that contained graded levels of nPP (0.53, 0.59, and 0.74%) and were used to determine a regression curve against which improvements in phosphorus utilization. based on tibia and/or toe ash, were measured. Performance measures were determined from 8 to 15 d of age. All birds were sampled at 16 d and the middle toe from both feet and the right tibia removed for drydefatted ash determination. PHY and CA affected (P<0.05) gain, feed consumption, feed-to-gain ratio, toe and tibia ash but no interactions were seen. When tibia and toe ash were regressed on diet nPP, linear regressions were obtained (29.11 + 44.28\*nPP,  $r^2=0.90$ , P<0.05; 0.45 + 34.38\*nPP, r<sup>2</sup>=0.88, P<0.05; respectively). Using these equations the sparing effect of nPP for the two feed additives was calculated. A main effect (P<0.05) of PHY and CA on nPP sparing effect was observed but no interactions were seen. The sparing effect, based on tibia ash, was 0.0280 and 0.0898% nPP for 300 and 600 FTU PHY/kg, respectively. CA had an effect (P<0.05) only when added to the diet at a 3% (0.031% nPP) level. A similar sparing effect was found for 3% CA and for 600 PHY when toe ash was used (0.031 and 0.0875% nPP, respectively). When 600 FTU PHY/kg and 3% CA were used together the sparing effect was 0.130 and 0.125% nPP for tibia and toe ash, respectively.

Key Words: Broilers, Citric acid, Phytase, Non-phytate phosphorus

554 The effects of supplemental phytase on egg shell quality in broiler breeder hens. M. Lilburn\* and J. Nixon, *The Ohio State University/OARDC*.

Environmental concerns coupled with legislative efforts aimed at minimizing nutrient excesses in animal manures have resulted in an array of dietary strategies to help achieve these goals. The enzyme phytase and its incorporation into commercial type diets has become one of these aforementioned tools. Phytase liberates phytate-bound phosphorus from plant source feedstuffs, ultimately resulting in greater dietary utilization of plant phosphorus and lower levels in the excreta. While there has been considerable literature on the phosphorus requirement of various classes of poultry and the role that phytase plays in meeting these requirements, there is still considerable confusion with respect to optimizing phosphorus nutrition and minimizing excretion. There has been considerable phytase research with commercial egg-type hens, but to date there is a paucity of research with broiler breeder hens. The objectives of the research reported herein was to study, in broiler breeder hens, the effects of different levels of available phosphorus(.12%, .22%, .32%) in diets with and without supplemental phytase (0,300 units). Mature broiler breeder hens were fed these diets (154 g/hen/d) beginning at 36 wk of age and the experimental periods were 36 to 40, 40 to 44, and 44 to 48 wk. Egg production was recorded daily. During the last wk of each production period, all eggs were individually weighed and broken out for shell wt determination. There was an increase in egg wt with age and an increase in shell wt with age and level of available P. The best results were observed with 0.22% AvP. At the end of the study, all eggs were divided into 5 classes with mean egg wts of 54.5 g, 58.9 g, 63.7 g, 67.8 g, and 72.6 g. Within each egg wt class, the 0.22% AvP treatment resulted in the highest shell wt and this would have allowed for 334 mg AvP per day. The effects of supplemental phytase were inconsistent.

Key Words: Phytase, Phosphorus, Broiler breeder

**555** Effect of dietary calcium and phosphorus levels on response of broiler chicks to phytase supplementation. F. Yan, J. H. Kersey, C. A. Fritts, and P. W. Waldroup\*, *University of Arkansas*.

It has been frequently demonstrated that addition of phytase to cornsoybean meal diets will improve the utilization of the phytate-bound P for broiler chickens. The effect of the phytase on release of other nutrients is less clear-cut. One phytase producer suggests that Ca is also released by phytase and that consequently the dietary Ca level might be reduced. A study was conducted to examine the effects of phytase supplementation on diets with varying levels of Ca with different levels of nonphytate P. A diet was formulated that provided nutrients in excess of NRC (1994) with 1.0% Ca and 0.5% nonphytate P. By varying the amounts of dicalcium phosphate and limestone in aliquots of a common basal diet, diets were prepared with 1) Low-P and Low-Ca; 2) Low-P and High-Ca; 3) High-P and Low-Ca; 4) High-P and High-Ca. The diets were analyzed to determine Ca and P content and blended as needed to provide test diets in a factorial arrangement of three Ca levels (0.5%, 0.7%, 0.9%) with eight levels of nonphytate P (0.15% to 0.5% in increments of 0.05%). These 24 diets were then divided and half supplemented with 1000 units/kg of phytase (Natuphos#, BASF). Each treatment was fed to six pens of six male broilers from one to 21 d of age. When diets received no phytase, both BW and tibia ash (TA) were depressed at the lowest level of P as Ca increased. Adding phytase to these diets improved both BW and TA, as a result of increased availability of P. At both 0.5% and 0.7% Ca, the dietary Ca level was a limiting factor in maximizing both BW and TA regardless of level of nonphytate P or phytase supplementation. Diets containing 0.9% Ca supported greater BW and TA than diets with lower Ca levels regardless of P level or phytase supplementation. These data indicate that minimal Ca appears to be released by phytase and that no reduction in Ca should be implemented when diets are supplemented with phytase.

Key Words: Phytase, Calcium release, Phosphorus

#### **556** Evaluation of dietary chlorine for turkey poults. K.D. Roberson<sup>\*1</sup>, <sup>1</sup>*Michigan State University*.

Dietary salt level has been linked to roundheart disease in turkey poults with dispute. Past research has focused on either added salt or specifically on sodium due to hypertensive effects associated with high dietary sodium. Problems with roundheart mortality have been common after 6 days of age in the turkey growout facility at Michigan State University when crumbles are fed. No roundheart mortality was observed in one trial when part of the sodium requirement was provided by sodium bicarbonate in place of sodium chloride in mash feed. A 14-day trial was conducted in a battery brooder to determine the effects of varying dietary chlorine levels on growth performance and roundheart mortality when dietary sodium was held constant at 0.17%. A corn-soybean meal-fish meal based diet was fed in a mash form to 6 pens of 8 toms per treatment. The calculated dietary chlorine levels were 0.17, 0.21, 0.25 or 0.29%. There was a quadratic (p=0.010) response of 14-d body weight to dietary chlorine as poults were lighter when 0.17% chlorine was fed  $(347~{\rm g})$  compared to poults fed 0.21 or 0.25% chlorine  $(370~{\rm or}~374~{\rm g},$ respectively). There was no effect on feed efficiency. Mortality was low in the trial and not due to roundheart. Heart weight, as a percentage of body weight, ranged from 2.92 to 3.25% as dietary chlorine increased and resulted in a linear response at p=0.080. The results of this study suggest that altering the dietary electrolyte balance by feeding chlorine as high as 0.29% does not induce roundheart mortality in turkey poults.

The NRC requirement for chlorine (0.15%) appears to be too low for 0 to 4-week-old poults.

Key Words: Chlorine, Poult, Roundheart

557 Influence of supplemental citric acid and sodium and potassium citrate on phytate-phosphorus utilization in broiler chicks fed phosphorus-deficient diets from one to 42 days of age. Ahmed Metwally\*, Animal Prod. Dept., Fac. of Agric., Assiut University, Assiut-Egypt..

Experiments were conducted to determine if the addition of citric acid to a phosphorus (P) deficient corn-soybean meal (SBM) diet would enhance phytate P utilization in broilers. Citric acid (C), Citric acid and

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**558** Influence of IBDV on the immune system and incidence of proventriculitis in SPF leghorns. T.V. Dormitorio<sup>\*1</sup>, J.J. Giambrone<sup>1</sup>, and K. Cookson<sup>2</sup>, <sup>1</sup>Auburn University, Auburn, Alabama, <sup>2</sup>Ft. Dodge Animal Health, Lawrenceville, Georgia.

Infectious bursal disease virus (IBDV) isolates have been linked to cases of proventriculitis in commercial broilers. These isolates appear to be variant in nature as they do not cause much edema, but mostly atrophy of the bursae. Some are very pathogenic and associated with immune suppressed flocks.

Fourteen-day old Specific Pathogen Free (SPF) white leghorns received four different IBDV vaccines. At 28 days, chicks were challenged with 707B IBDV isolate by eye and nose routes. At 34 days of age, pullets were tested for cell-mediated immune (CMI) response using a skin test. At 36 days of age all birds were killed and weighed, and the bursa were weighed and examined post mortem for gross lesions. Bursa weight to body weight ratios were also determined and the means of all groups analyzed for differences using SAS system.

SPF white leghorns were susceptible to the 707B IBDV field isolate at 26 days of age. Nearly 100% of these birds had severe gross lesions in bursa (atrophy) and proventriculus (glandular enlargement and edema), and numerically reduced final body weight. All commercial live intermediate IBDV vaccines produced excellent protection against proventricular lesions induced by this isolate. This IBDV isolate also cause a reduced CMI response. These data provide more evidence that this IBDV isolate had a major role in causing immunosuppression and proventriculitis in chickens.

Key Words: IBDV, Proventiculitis, CMI

## $559~~In~ovo~administration~of~experimental reovirus vaccines^b. Z.Y. Guo* and J.J. Giambrone, Auburn University, Auburn AL .$

Avian reoviruses are an important cause of poultry diseases worldwide and can induce various diseases in chickens. *In ovo* administration of viral vaccines is a new technique used in preventing diseases. It is commonly used for the administration of Marek's Disease and infectious bursal disease viruses (IBDV) vaccines in commercial broilers. According to our preliminary experiments, the current reovirus vaccine, Enterovax, complexed (combined) with specific antibody against reovirus was too pathogenic for *in ovo* use. Therefore, a milder reovirus vaccine, Chick Syno-vac, was employed. This vaccine, complexed with antibody, was able to induce immunity against reovirus challenge and was less pathogenic than Enterovax. We are currently examining an even milder vaccine (VA Chick Vac) combined with antibody to determine its pathogenicity and immunogenicity, when given by *in ovo* route.

Key Words: Reovirus ,  $In \ ovo,$  Vaccination

**560** Changes in serum levels of ovotransferrin during experimental inflammation and diseases in chickens. H. Xie<sup>\*1,2</sup>, N. Rath<sup>1</sup>, F. Clark<sup>2</sup>, L. Newberry<sup>2</sup>, W. Huff<sup>1</sup>, G. Huff<sup>1</sup>, and J. Balog<sup>1</sup>, <sup>1</sup>PPPSRU, ARS, USDA, <sup>2</sup>Department of Poultry Science, University of Arkansas.

We have identified serum ovotransferrin as an avian acute phase protein. To measure the changes in serum levels of ovotransferrin during inflammation and poultry diseases, we developed a solid phase competitive Sodium citrate(1:1 mixture,CS) and Citric acid and Sodium citrate and Potassium citrate (1:1:1 mixture, CSP) were added at levels of 0, 4.5 and 6% to a P-deficient diet containing 0.91 Ca and 0.22% available phosphorus. Each of three dietary treatments was fed to replicate groups of eight chicks from one to 42 days of age. Increasing levels of dietary citrate to 4.5% increases performance traits. Carcass quality and tibia weight and ash improved for chicks fed 4.5% mixture of CSP. The bone ash response to the mixture of CSP was much greater than the bone ash response to the mixture of CS. The results of this study indicate that the mixture of CSP at level of 4.5% enhances phytate P utilization in broiler diets from one to 42 days of age.

Key Words: Citrate, Performance, Phytate-phosphorus utilization, chicks

enzyme immunoassay using rabbit anti-chicken serum transferrin antibody and biotinylated ovotransferrin. Serum ovotransferrin competes with biotinylated ovotransferrin to bind to anti-chicken transferrin antibody. The residual biotinylated ovotransferrin bound to anti-chicken transferrin is then detected using streptavidin-horseradish peroxidase followed by a colorimetric detection step. Serum levels of ovotransferrin are then determined according to a standard curve generated using known concentrations of ovotransferrin. Inflammation was experimentally induced in 4-wk-old male broiler chickens by subcutaneous injection of croton oil, and specific pathogens free (SPF) chickens were challenged with various bacteria or viruses to induce specific disease. In the experimental inflammation model with croton oil, the serum levels of ovotransferrin increased by 16 h post-injection, reached a peak by 72 h, remained high through 5 days, and returned to the basal level of olive oil-injected sham-controls by 10 days. Similarly, compared to the control birds, SPF chickens challenged with E.coli, pox virus, reovirus, infectious bursal disease virus, or laryngotracheitis virus had significantly higher levels of ovotransferrin in serum (P < 0.05). Our results demonstrate that ovotransferrin can be utilized as a clinical marker for inflammation associated with certain infectious avian diseases.

Key Words: Ovotransferrin, Inflammation, Enzyme immunoassay

**561** Differential intestinal response to *Eimeria acervulina* challenge in broiler chickens. B.C. Morris<sup>\*1</sup>, H.D. Danforth<sup>2</sup>, D.J. Caldwell<sup>3</sup>, and A.P. McElroy<sup>1</sup>, <sup>1</sup>Virginia Tech, Blacksburg, VA, <sup>2</sup>USDA/ARS/LPSI/PBEL, Beltsville, MD, <sup>3</sup>Texas A&M University, College Station, TX.

Immunovariability between coccidial species in vaccines and those found in poultry rearing facilities has emerged as a potential complication associated with vaccination. The host pathogen interaction and immune response in the intestine must be further investigated to understand immunity and pathophysiology to Eimeria in chickens. Experiments were conducted comparing two isolates of Eimeria acervulina (EA), EA1 and EA2. In three experiments, commercial broilers chicks were divided into control (non-challenged) and EA1 or EA2 challenged (14 days of age) groups. In all 3 experiments, EA1 resulted in significantly (P<0.05) higher lesion scores than EA2, however, weight gain of EA1 challenged birds was not significantly different from controls. EA2 challenged birds had significantly higher lesion scores than control birds in Expts 1 and 3, with no lesions characteristic of classical EA infection in Expt 2. EA2 resulted in significantly decreased weight gain as compared to EA1 or control in Expt 3. While EA1 resulted in classical EA lesions with no significant difference in weight gain, EA2 resulted in few classical lesions with significant depression of weight gain. Subjective observation of intestines from EA2 challenged birds was suggestive of a severe secretory intestinal response and weakened intestinal strength. In Expt 4, EA2 oocysts were cleaned with 5.25% sodium hypochlorite to evaluate the possibility of an external bacterial factor contributing to the observed detrimental affects in the presence of few lesions. Birds were challenged with bleached or non-bleached EA2. Although there was no significant difference in lesion scores between EA2 challenged groups, non-bleached EA2 resulted in significantly decreased weight gain