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Impacts of Phytase and/or Carbohydrases on Performance, Intestinal Organs and Bone Development in Broilers Fed Wheat-Based Diets Containing Different Levels of Phosphorus

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Abstract: The experiment was conducted to study the effects of an enzyme cocktail (CGX) (consist of cellulase, β-glucanase and xylanase) and/or phytase supplementation to wheat based broiler starter diet on live performance, intestinal organ measurements and bone development. One-day-old male Ross 308 strain broiler chickens (n = 120) were allocated to 8 dietary treatments in a randomized complete block design. The treatments were as follows: Positive control (containing 0.50% Nonphytate Phosphorus (NPP)) (PC), PC + Phytase (150 g ton^{-1} feed) (PCP), PC + CGX (100 g ton^{-1} feed) (PCR), PC + CGX + phytase (PCRP) and negative control (containing 0.25% NPP) (NC), NC + phytase (150 g ton^{-1} feed) (NCP), NC + CGX (100 g ton^{-1} feed) (NCR), NC + CGX + Phytase (NCRP). The experiment lasted for 21 days. Birds fed the PC diet exhibited significantly higher Weight Gain (WG) ($p<0.001$) and improved Feed Conversion Ratio (FCR) ($p<0.001$). Birds fed the NC diet exhibited significantly ($p<0.01$) higher duodenum, jejunum, ileum and cecum length. Combining two enzymes significantly ($p<0.05$) reduced the relative length of duodenum, jejunum, ileum and cecum in NC diets. Nonphytate phosphorus level was significantly affected ($p<0.01$) tibia length of birds, although enzymes was not influenced statistically to tibia length or width. There was a significant increase ($p<0.01$) in the percentage of toe ash with increasing NPP level at 21 days. Toe ash was not affected by phytase, CGX or in combination. Thus, supplementation of phytase and CGX enhanced broiler chick performance with respect to WG and FCR.

Key words: Broiler, carbohydrase, bone development, performance, phytase, wheat

INTRODUCTION

Wheat has not been commonly used in poultry diets because of the presence of antinutritional factors consisting mainly of water-soluble and nonsoluble Nonstarch Polysaccharides (NSP) compared to maize. However, wheat can be more cost effective feed ingredients compared to maize as the major grain in broiler diets in various regions of the world. The most important NSP fraction in wheat has been reported to be xylans and β-glucans (Leeson and Summers, 1991). These substances have been found to adversely effect nutrient digestion, absorption and gut microflora, by increasing the viscosity of digesta in the gut (Hesselman and Aman, 1986; Choct *et al.*, 1992). High levels of wheat inclusion into wheat-based broiler diets had unfavourable effects on broiler performance. Studies have shown that these viscous, indigestible polysaccharides cause an enlargement of digestive organs such as the pancreas because they produce more intestinal enzymes to compensate for the impediment of digestion of feed and absorption of nutrients (Ikegami *et al.*, 1990). These problems can be resolved by limiting the quantity of wheat in the diet, or possibly by adding synthetic exogenous enzymes to the feed that includes wheat (Leeson *et al.*, 1996).

Xylanase and β-glucanase are enzymes that degrade NSP (Choct *et al.*, 2004) and have been shown to improve the nutritive value of wheat and barley-based diets for birds (Cowieson *et al.*, 2005; Juanpere *et al.*, 2005; Meng *et al.*, 2005) by reducing the antinutritional effects of nonstarch polysaccharides (Choct *et al.*, 2004).

On the other hand, approximately 2/3 of the Phosphorus (P) in cereal grains are present in the form of phytic acid (Akyurek *et al.*, 2005). The ability of poultry to use phytate P is poor (Wu *et al.*, 2004) due to insufficient quantities or lack of intestinal phytase secretion. This inadequacy of poultry to use phytate P results in the excretion of large amounts of P in the manure, posing an environmental concern especially, in areas of intensive animal production (Wu *et al.*, 2004). Microbial phytase is commercially available for use in poultry feeds. This

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enzyme has been shown to increase the utilization of phytate from broiler feedstuffs (Kornegay *et al.*, 1996).

Calcium and phosphorus are primary inorganic nutrients in the bone that may be important for bone health and strength (Rath *et al.*, 2000). Nelson *et al.* (1971) reported that the addition of phytase to broiler diets resulted in a marked improvement of the utilization of phytate phosphorus as measured by bone ash. Similarly, several researchers have shown that dietary supplementation of microbial phytase improved the availability of Ca and P (Sohail and Roland, 1999).

There is little information on the effectiveness of NSP degradation enzymes and phytase complexes for chickens fed with wheat diets. Therefore, this study was carried out mainly to test the effects of a enzyme cocktail (CGX) of cellulase, β-glucanase and xylanase or phytase individually or in combination on growth performance, intestinal organ measurements and bone development in broiler fed on wheat-based diets containing adequate or deficient Nonphytate Phosphorus (NPP).

MATERIALS AND METHODS

Animals and housing: Male Ross 308 broilers (1 day old chicks, n = 120) were obtained from a local parent stock supplier and randomly transferred to compact-type three tier cages, three chicks per cage. Battery cages were equipped with wire mesh, dropping trays, nipple drinkers and trough feeders. The battery cages were placed in an environmentally controlled room with windows. The experiment consisted of 8 dietary treatments and was set up in a completely randomized design, in which 15 chicks were randomly assigned to each of the eight treatments, five replicates each. The experiment lasted for 21 days and the chicks were fed the experimental diets throughout the experimental period. Chicks had free access to feed and water. The lighting regime was 23 h day^{-1}. Birds were weighed by pen at 1, 7, 14 and 21 days of age.

Diets: The basal components of all diets were wheat, soybean meal, maize gluten meal, full fat soybean meal, soy oil and mineral and vitamin premix supplement, from a local feed market. Two different levels of NPP were adjusted (Positive Control (PC) 0.50% NPP and Negative Control (NC) 0.25% NPP) and two different commercial enzyme preparations (Ronozyme® P5000 and Roxazyme® G2 G (CGX)) were used. Phytase (Ronozyme® P5000, DSM Nutritional Products Ltd., Basel, Switzerland) containing 5000 FYT g^{-1} and a enzyme complex derived from *Trichoderma longibrachiatum* (Roxazyme® G2 G, DSM Nutritional Products Ltd, Basel, Switzerland) containing cellulase (endo-1,4-β-glucanase; EC 3.2.1.4); β-glucanase (endo-1,3(4)-β-glucanase; EC3.2.1.6) and xylanase (endo-1,4-β xylanase; EC 3.2.1.8), were used. Dietary treatments were: Positive Control (containing 0.50% NPP) (PC), PC + Phytase (150 g ton^{-1} feed) (PCP), PC + CGX (100 g ton^{-1} feed) (PCR), PC + CGX + phytase (PCRP), Negative Control (containing 0.25% NPP) (NC), NC + Phytase (150 g ton^{-1} feed) (NCP), NC + CGX (100 g ton^{-1} feed) (NCR) and NC + CGX + Phytase (NCRP). Experimental diets were formulated using ration-formulation software (UFFDA, University of Georgia, 1992, Athens, GA) to be isocaloric and isonitrogenous following National Research Council recommendations (NRC, 1994). Experimental diets were formulated to contain 22% crude protein and 12.67 MJ ME kg^{-1} and other essential nutrients (Table 1). Birds were fed the experimental diets *ad libitum* in mash form. Feed intake was recorded weekly. The Feed Conversion Ratio (FCR) was calculated as grams of feed consumed per chick divided by grams of Weight Gain (WG) per chick.

Table 1: The ingredients and chemical composition of experimental basal diets (as-fed basis)

	Treatments	
Ingredient (g kg^{-1} diet)	NC	PC
Wheat	606.18	597.39
Soybean meal (44% CP)	226.80	227.74
Maize gluten meal (60% CP)	35.81	36.71
Full-fat soybean (37.5% CP)	75.00	75.00
Soybean oil	15.73	18.32
Monocalcium phosphate	5.07	16.57
Limestone	24.90	17.76
Salt	2.95	2.95
Vitamin-mineral premix[1]	2.50	2.50
L-Lysine	3.51	3.51
DL-methionine	1.55	1.55
Total	1000.00	1000.00
Calculated nutrient content[2]		
ME, MJ kg^{-1}	12.67	12.67
Crude protein (%)	22.00	22.00
Ether extract (%)	4.29	4.53
Linoleic acid (%)	1.84	2.01
Crude fiber (%)	3.10	3.08
Crude ash (%)	6.50	6.78
Calcium (%)	1.05	1.05
Nonphytate phosphorus (%)	0.25	0.50
Total phosphorus (%)	0.45	0.70
Sodium (%)	0.16	0.16
Clorine (%)	0.23	0.23
Lysine (%)	1.43	1.43
Methionine (%)	0.51	0.51
Methionine + cystine (%)	0.88	0.88
Tryptophan (%)	0.24	0.24

[1]Provided per kilogram of diet: vitamin A, 8,000 IU (as retinyl acetate); vitamin D_3, 2,500 IU (as cholecalciferol); vitamin E, 30 mg (as α-tocopheryl acetate), vitamin K_3, 2.5 mg (as menadione sodium bisulfite), vitamin B_1 2 mg (thiamine), vitamin B_2, 5 mg (as riboflavin), vitamin B_6, 2 mg (as pridoxamine), vitamin B_{12}, 0.01 mg (as cyanocobalamin), niacin, 30 mg, calcium-D-pantothenate, 8 mg, folic acid, 0.5 mg, D biotin, 0.045 mg, choline chloride, 300 mg, vitamin C, 50 mg, MnO_2, 70 mg, $FeSO_4\_7H_2O$, 35 mg, ZnO, 70 mg, $CuSO_4\_5H_2O$, 8 mg, Ca $(IO_3)_2$, [2]Based on NRC (1994) values for feed ingredients

Gastrointestinal tract and some internal organs measurements: At 21 days of age, two birds taken randomly from each cage (10 birds per treatment) were starved overnight and killed by cervical dislocation. The birds were then weighed and their organs harvested. Organ analyses included heart, liver, spleen, bursa fabricious, thymus, crop, proventriculus, gizzard, pancreas, duodenum, jejunum, ileum and cecum weights and duodenum, jejunum, ileum and cecum lengths. The GI tract was portioned into crop, proventriculus, gizzard, pancreas, duodenum, jejunum, ileum and ceca. The ileum, defined as the region from Meckel' s diverticulum to a point 40 mm proximal to the ileocecal junction. The jejunum was defined as the portion of intestine extending from the bile duct enterance to Meckel' s diverticulum. All organ weights and lengths were expressed as a percent of body weight.

Bone development measurements: Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end (Wu *et al*., 2004). The left and right middle toes of birds were removed and frozen for subsequent determination of toe ash percentage. The toe samples were dried at 105°C and then ashed in muffle furnace at 600°C overnight and the ash percentage was calculated considering the weight. The right tibia was removed and cleaned of adhering tissue. The tibia was measured (length and width).

Statistical analyses: Collected data were recorded on a weekly basis and statistically subjected to ANOVA using a statistical package program (SAS Institute, 1994). The differences between group means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Performance: The result of the growth performance is shown in Table 2. Birds fed the PC diet exhibited significantly higher WG ($p<0.001$) and improved FCR ($p<0.001$). The addition of CGX alone did not affect WG, but when phytase alone or in combination with CGX were added, there was a numerical increase in WG compared to NC diet. However, it should be noted that this numerical increase might have been influenced by the relatively higher feed intake. Weight gain and feed intake were higher ($p<0.001$) in the chicks on the PC diet than those on the NC diet. However, supplementation with CGX and phytase had no significant effect on FCR.

As discussed in this study deficiencies of NPP decreased WG, FI and FCR in chicks (Skinner and Waldroup, 1992; Yan *et al*., 2001). The addition of phytase was a numerically improved in WG, FI and FCR. There are several studies, which indicate that microbial

Table 2: Effect of treatments on broiler performance (0-21 days of age)

Diets	Weight gain (g bird^{-1})	Feed intake (g bird^{-1})	FCR
PC	751.1^{c}	1046.9^{cd}	1.394^{a}
PCP	764.0^{c}	1064.3^{cd}	1.393^{a}
PCR	753.8^{c}	1091.5^{d}	1.448^{ab}
PCRP	723.2^{c}	1037.7^{cd}	1.435^{ab}
NC	566.5^{ab}	901.8^{ab}	1.592^{c}
NCP	594.9^{ab}	895.3^{ab}	1.505^{abc}
NCR	535.4^{a}	818.0^{a}	1.528^{bc}
NCRP	632.3^{b}	961.3^{bc}	1.520^{bc}
SEM	17.00	18.44	0.02
Source of variation P			
NPP level	<0.001	<0.001	<0.001
Enzymes (E)	0.618	0.648	0.658
P×E	0.210	0.059	0.419

n = 5 per treatment, $^{a-d}$Means in the same column with different superscripts differ significantly ($p<0.05$)

Table 3: Effect of dietary treatments on relative heart, liver, spleen, bursa fabricious and tymus weights (g/100 g BW) of birds at 21 day of age

Diets	Heart	Liver	Spleen	Bursa fabricious	Tymus
PC	0.680^{a}	2.460^{a}	0.090	0.300^{ab}	0.620
PCP	0.680^{a}	2.750^{ab}	0.100	0.300^{ab}	0.620
PCR	0.710^{ab}	2.680^{ab}	0.100	0.260^{a}	0.550
PCRP	0.680^{a}	2.920^{bc}	0.120	0.320^{ab}	0.600
NC	0.760^{ab}	2.920^{bc}	0.110	0.300^{ab}	0.610
NCP	0.910^{c}	2.880^{bc}	0.090	0.390^{b}	0.600
NCR	0.800^{b}	2.580^{ab}	0.130	0.340^{ab}	0.550
NCRP	0.960^{c}	3.180^{c}	0.110	0.270^{a}	0.520
SEM	0.020	0.049	0.004	0.011	0.020
Source of variation P					
NPP level	<0.01	0.021	0.495	0.121	0.495
Enzymes (E)	0.045	0.003	0.306	0.323	0.613
P×E	0.019	0.102	0.149	0.061	0.930

n = 5 per treatment, $^{a-c}$Means in the same column with different superscripts differ significantly ($p<0.05$)

phytase supplementation increases body weight gain, feed intake and feed efficiency in broiler chickens (Simons *et al*., 1990; Broz *et al*., 1994; Kornegay *et al*., 1996; Biehl and Baker, 1997; Sebastian *et al*., 1997; Wu *et al*., 2003; Driver *et al*., 2005). However, some researchers reported that the addition of phytase to corn-soybean meal based diet did not improve body weight gain or feed intake in broiler chickens (Ibrahim *et al*., 1999; Waldroup *et al*., 2000; Yan *et al*., 2003).

On the other hand, the results of this experiment indicated that the CGX and phytase combination slightly improved broiler live performance when it was added into wheat-based diet. Positive results observed with NSP degrading enzymes supplementation into wheat-based diet were probably associated with digesta viscosity as previously reported by Hesselman and Aman (1986), Choct *et al*. (1992, 1995), Adeola and Bedford (2004) and Cowieson *et al*. (2005).

Effect of dietary treatments on intestinal organ measurements: Effect of phytase and CGX, individually or in combination on the relative weight and length of different sections of intestinal organs are presented in Table 3-5. The treatments had no effect ($p>0.05$) on the

Table 4: Effect of dietary treatments on Gastrointestinal Tract (GIT) weights (g/100 g BW) of birds at 21 day of age

Treatments	Crop	Proventriculus	Gizzard	Pancreas	Duodenum	Jejunum	Ileum	Cecum
PC	0.320^{b}	0.590^{ab}	2.480^{b}	0.360	1.010	1.850^{ab}	1.350	0.520^{a}
PCP	0.270^{ab}	0.530^{ab}	2.340^{ab}	0.340	0.950	2.000^{ab}	1.410	0.660^{ab}
PCR	0.250^{a}	0.620^{b}	2.500^{b}	0.330	1.080	1.980^{ab}	1.510	0.610^{ab}
PCRP	0.290^{ab}	0.610^{ab}	2.370^{ab}	0.330	0.980	1.910^{ab}	1.550	0.660^{ab}
NC	0.320^{b}	0.530^{ab}	3.140^{c}	0.350	1.080	2.030^{b}	1.680	0.750^{b}
NCP	0.290^{ab}	0.540^{ab}	2.130^{ab}	0.330	1.070	1.680^{a}	1.450	0.640^{ab}
NCR	0.320^{b}	0.530^{ab}	2.400^{b}	0.350	0.990	1.710^{ab}	1.500	0.780^{b}
NCRP	0.320^{b}	0.500^{a}	1.960^{a}	0.350	0.900	1.720^{b}	1.360	0.620^{ab}
SEM	0.008	0.013	0.068	0.006	0.023	0.039	0.035	0.024
Source of variation P								
NPP level	0.048	0.015	0.892	0.738	0.883	0.046	0.551	0.057
Enzymes (E)	0.191	0.711	<0.010	0.509	0.430	0.636	0.807	0.789
P×E	0.346	0.314	0.002	0.567	0.269	0.084	0.091	0.093

n = 5 per treatment, $^{a-c}$Means in the same column with different superscripts differ significantly ($p<0.05$)

Table 5: Effect of dietary treatments on Gastrointestinal Tract (GIT) length (cm/100 g BW) of birds at 21 day of age

Treatments	Duodenum	Jejunum	Ileum	Cecum
PC	3.190^{abc}	7.580^{ab}	7.580^{ab}	1.630^{a}
PCP	2.970^{a}	6.890^{a}	7.010^{a}	1.570^{a}
PCR	3.190^{abc}	7.530^{ab}	7.430^{ab}	1.800^{abc}
PCRP	3.020^{a}	7.430^{ab}	7.690^{ab}	1.680^{ab}
NC	3.820^{cd}	10.090^{c}	10.320^{c}	2.150^{c}
NCP	3.770^{bcd}	8.740^{bc}	9.020^{bc}	1.900^{abc}
NCR	3.880^{d}	9.570^{c}	9.380^{c}	2.050^{bc}
NCRP	3.120^{ab}	7.450^{ab}	7.420^{ab}	1.670^{ab}
SEM	0.089	0.230	0.240	0.051
Source of variation P				
NPP level	<0.010	<0.010	<0.010	0.005
Enzymes (E)	0.135	0.019	0.059	0.159
P×E	0384.000	0.061	0.032	0.219

n = 5 per treatment, $^{a-d}$Means in the same column with different superscripts differ significantly ($p<0.05$)

Table 6: Mineralization parameter of tibia and toe at 21day of age

Treatments	Tibia length (mm)	Tibia width (mm)	Toe ash (%)
PC	70.340^{ab}	5.580	12.19^{c}
PCP	71.040^{b}	6.040	12.18^{c}
PCR	69.820^{ab}	5.900	11.89^{c}
PCRP	70.800^{b}	5.920	11.72^{c}
NC	65.900^{a}	5.080	9.74^{b}
NCP	65.700^{a}	5.880	8.07^{a}
NCR	66.000^{a}	5.240	8.72^{ab}
NCRP	68.660^{ab}	5.940	8.92^{ab}
SEM	0.592	0.117	0.301
Source of variation P			
NPP level	<0.010	0.165	<0.010
Enzymes (E)	0.619	0.176	0.310
P×E	0.749	0.711	0.335

n = 5 per treatment, $^{a-c}$Means in the same column with different superscripts differ significantly ($p<0.05$)

relative weight of the spleen, thymus, pancreas, duodenum and ileum. The relative weight of hearth, liver, bursa fabricious and crop were lower in PC groups when compared to the NC groups. Birds fed the NC diet exhibited significantly ($p<0.01$) higher duodenum, jejunum, ileum and cecum length. Combining two enzymes significantly ($p<0.05$) reduced the relative length of duodenum, jejunum, ileum and cecum in NC diets. Additions of phytase, CGX and the enzyme combination reduced the relative length of the duodenum, jejunum, ileum and cecum in PC diets, but the differences were not significant ($p>0.05$). In addition to, supplementation of CGX numerically reduced the relative weight of duodenum, jejunum and ileum.

In this experiment, birds fed diets containing deficient NPP had heavier digestive tract and also phytase and CGX combination was decreased intestinal organ weight and length. The relative weights of the intestinal organs were lower in using phytase and CGX combination at NC and PC diets when compared to the control groups. The relative length of the duodenum, jejunum, ileum and cecum was reduced by phytase and CGX combination supplementation. This effect was reflected in increased WG and improvement in FCR. Enhanced degratation of non-starch polysaccarides by enzyme supplementation is probably reflected in the gastrointestinal organ weight and length.

Gastrointestinal tract weight and length could be improved by increased digestion of the NSP. For example, the addition of a β-glucanase to a barley-based diet was shown by Viveros *et al*. (1994) to improve weight gain and to reduce to relative lengths of small intestinal regions and the ceace.

Bone development: The result of the bone development is shown in Table 6. Tibia width of birds at 21 days was not affected significantly by the NPP level of the diet. In experiment, NPP level was significantly affected ($p<0.01$) tibia length of birds, although enzymes was not influenced statistically to tibia length or width. There was a significant increase ($p<0.01$) in the percentage of toe ash with increasing NPP level at 21 day. Toe ash was not affected by phytase, CGX or in combination. In experiment, NPP levels affected chick toe ash, although enzymes were not influenced. Therefore, toe ash effectively reflected the response in mineralization to different dietary NPP levels and it could potentially be used to quantity bone mineralization status in birds. Although, toe ash was lower in birds fed lower NPP, there appeared to be adequate bone mineralization since there were no leg problems observed in these birds and toe ash values were all in the adequate range.

In experiment, there was a significant increase in percentage of toe ash with increasing dietary NPP. This findings is not surprising because the percentage of toe ash is highly dependent on the amount of NPP in diet. Analogous findings have also been reported in previous trials (Ledoux *et al*., 1995; Keshavarz, 2000). Although, toe ash was lower in birds fed lower NPP, there appeared to be adequate bone mineralization since there were no leg problems observed in these birds and toe ash values were all in the adequate range. It can be concluded that adequate NPP concentration and phytase were improving bone mineralization and increasing phosphorus digestibility.

Toe ash percentage was not worthwhile, improved by phytase supplementation in adequate or deficient diets. However, the effects of NPP level on toe ash percentage was more pronounced when NPP was reduced in the diet.

Tibia length and width were slightly affected by NPP level of the diet. This findings in line with the findings of Skinner and Waldroup (1992).

Few interactions between phytase and CGX were found, when these enzymes were added to wheat-based diet. In general, the interactions were positive.

CONCLUSION

Consequently, the present study suggested that the performance and bone mineralization of broilers fed wheat based diets were adversely affected when dietary NPP levels were lowered below NRC recommendations. These negative effects were restored by the addition of phytase with/without CGX. Birds fed diets containing deficient NPP had heavier digestive tract and also phytase and CGX combination was decreased intestinal organ weight and length.

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