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Comparison of Wheat Bran Phytase and a Commercially Available Phytase on Turkey Tom Performance and Litter Phosphorus Content

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Abstract: This study evaluated the effects of wheat bran phytase or a commercially available phytase (Natuphos® 600) on growth performance and skeletal integrity of toms and forms of litter phosphorus. Five-wk-old Hybrid Converter toms were fed corn-soybean meal based mash diets for 12 wk in four 3-wk phases. Treatments consisted of 1) control [0.50, 0.44, 0.38 or 0.35% nonphytate phosphorus (nPP) at 5-8, 8-11, 11-14, or 14-17 wk of age, respectively]; 2) low phosphorus (0.1 percentage units less nPP and 0.2 percentage units less calcium than treatment 1); 3) calcium and nPP same as 2, but with 3.27% wheat bran in diet (900 units/kg phytase activity); 4) calcium and nPP same as 2, but with Natuphos® added to provide 900 units/kg from 5 to 11 wk or 600 units/kg from 11 to 17 wk. Body weight and feed intake were measured at the end of each phase and bone fracture force, strength and ash and litter phosphorus (total and soluble) were measured at 17 wk. Body weight was higher when toms were fed the control diet throughout the study compared to all other treatments and was not affected by phytase source. Tibia fracture force and ash were decreased by the low phosphorus diet but was similar for both phytase sources compared to the control diet. Litter soluble phosphorus content was higher in treatment 4 than treatment 2, but not treatment 1. Feeding wheat bran phytase yielded similar bird responses and litter soluble phosphorus as Natuphos® when fed to commercial toms.

Key words: Phosphorus, phytase, turkey, wheat bran

Introduction

The use of phytase to improve phytate phosphorus utilization of feedstuffs such as corn and soybean meal to poultry has been studied with poults (Ravindran *et al*., 1995; Qian *et al*., 1996) and older turkeys (Ledoux *et al*., 1995; Atia *et al*., 2000, Rodehutscord *et al*., 2003). These studies utilized a microbial source of phytase to improve the availability of phosphorus (P) in plant feedstuffs to turkeys typically in the form of 3-phytase activity from a commercial fungal source. This type of phytase initiates hydrolysis of phytate at the phosphate attached to the number 3 carbon of inositol.

Some feedstuffs contain considerable 6-phytase activity (i.e., wheat, wheat bran, rye and barley), whereas others have little or no phytase activity (i.e., corn, oats, sorghum and oilseeds; Eeckhout and de Paepe, 1994). Phytase activity in grains such as wheat have a high correlation with overall P retention in pigs and broilers (r = 0.83; Barrier-Guillot *et al*., 1996b). Within wheat samples, the phytase activity can be highly variable (915 to 1581 phytase units/kg; Eeckhout and de Paepe, 1994). Much of this difference can be explained through cultivar differences (Barrier-Guillot *et al*., 1996a).

The objective of this study was to evaluate the effects of phytase source (commercial fungal 3-phytase or plant) needed during the growout of toms to maintain growth performance and bone integrity when dietary nPP is decreased by 1.0 g/kg and to measure the amount of soluble P content in the litter when phytase is fed. Serum pyridinoline was also measured to evaluate the potential usefulness of this marker of bone turnover status as a noninvasive test to evaluate bone integrity.

Materials and Methods

A total of 800 male poults (Hybrid Converter) were obtained from a commercial hatchery[1] and raised to 5 wk of age. The toms were fed a mash diet that met NRC (1994) recommendations for calcium and nPP and met or exceeded NRC (1994) recommendations for all other nutrients. At 5 wk of age, birds were weighed individually and sorted to equalize body weights across treatments. Average starting body weight across pens was 1.74 kg. There were approximately 25 toms/pen at the beginning of the 13-wk trial (8 pens per diet). The pens were 2.48 m X 3. 08 m and there was one hanging feeder and one bell waterer in each pen. Dietary treatments consisted of : 1) a control diet that provided adequate calcium and nPP for growth and bone strength in mash feed according to the report of Roberson (2003) (Table 1), 2) a negative control diet that was formulated to contain 0.1 percentage units less nPP and 0.2 percentage units less calcium than the control diet (dietary calcium was

decreased to prevent exacerbation of a phosphorus deficiency by widening the calcium:nPP ratio), 3) the same calcium and nPP as treatment 2, but with 3.27% wheat bran[2] in the diet to provide 900 units/kg phytase activity [phytase activity of the wheat bran was measured prior to feed mixing (Chen, 1996)], and 4) the same calcium and nPP as treatment 2, but with 900 units of analyzed phytase activity/kg diet from 5-11 wk of age or 600 units of analyzed phytase activity/kg from 11-17 wk of age from a commercial source[3] added to the diet. These levels of the commercial source have been used in turkey formulations in the U.S. Analysis of the premix by the manufacturer showed that the actual content of phytase activity in the premix was about 30% higher than the minimum guarantee of 600 units/g.

Body weight and feed consumption were measured at 8, 11, 14 and 17 wk of age. Gain of birds that died or were culled due to incorrect sex, pendulous crop, or leg injury was included for feed conversion calculations. Blood samples were taken via the wing vein from 8 birds (1 per pen) per treatment at 16 wk of age for determination of serum pyridinoline concentration as previously described (Lang *et al.*, 2001). At 17 wk of age, three toms were selected from each pen based upon average body weight and slaughtered at the Michigan State University Meat Laboratory. The left ulna, femur and tibia were excised and stored in a freezer for bone strength analysis. The right femur was also saved as thigh meat was deboned during the cut-up process in the meat laboratory. The bones were first evaluated for cross sectional area by computed tomography (Hathcock and Stickle, 1993) at the midshaft of the bones where impact would be applied to test fracture force. Thawed bones were tested for maximum fracture force with an Instron testing machine[4]. The tibiae were broken by the three-point bend method and the other bones were broken by the shear block method (ASAE, 1999). The three-point bend method is more appropriate for bones that have a length to width ratio of at least 10:1. The average length to width (midshaft) ratio of tibiae in this trial was about 13:1. The tibiae were placed on the fulcra so that only the flat portion (shaft) of the bone was between the fulcra. Hence, the length:width ratio of the tibiae between the fulcra was about 7:1. The broken pieces of bone were fat extracted and ashed according to AOAC (2000) procedures to determine bone mineralization.

Litter samples were collected at the end of the trial. Samples were taken from five points in each pen. A sample was taken from the middle of the pen which was between the feeder and waterer and four samples were taken from each corner of the pen about 30 cm from the wall or plastic screen divider between pens. Litter samples were mixed for each pen and placed in cold storage at 0°C. After one wk, sub-samples of the litter for each pen were transported to Purdue University for analysis of total P (Sands *et al.*, 2001) and water-soluble P content (Self-Davis and Moore, 2000).

The data were analyzed using the PROC GLM procedure of SAS (SAS Institute, 2001) using pen was the experimental unit. Differences between treatments were determined using the Student-Newman-Kuels test when $P<0.05$.

Results

Body weight at 8 wk of age was decreased ($P<0.001$) by the low nPP diet compared to control (adequate nPP) diet and persisted throughout the experiment (Table 2). Body weight remained lower than the control diet when either source of phytase was added to the low nPP diet. Birds fed Natuphos® were heavier at 11 wk of age compared to the low nPP diet and birds fed wheat bran were heavier at 17 wk than the birds fed low nPP. There were no significant differences in body weight of toms fed either source of phytase at any age in the experiment. Feed conversion was lower ($P<0.001$) for the control diet than the low P diet at 8 wk of age and this response persisted until the end of the trial ($P<0.02$). Addition of phytase to the low phosphorus diet did not improve feed conversion compared to the low P diet. However, at 17 wk of age the feed conversion of birds fed wheat bran phytase was not significantly different than birds fed compared to the control diet.

Litter P was reduced ($P<0.01$) by the low nPP diet compared to the control diet. Litter from birds fed the low nPP diet with phytase had similar litter P concentrations observed for birds fed the low nPP diet. Litter dry matter averaged 53.43% and was not significantly affected ($P = 0.588$, SEM = 0.74) by dietary treatment (data not shown). The amount of soluble P in the litter was higher ($P<0.05$) when Natuphos® was fed compared to when the low nPP diet was fed without phytase, but was not different from wheat bran or adequate nPP fed birds. When expressed as a percentage of total P, the amount of soluble P in the litter was increased ($P<0.01$) when either source of phytase was fed compared to adequate nPP fed birds, largely due to a reduction in total P in litters from birds fed either source of phytase. Birds fed Natuphos® also had higher soluble P as a percent of total P in the litter compared to birds fed the low P diet without phytase.

Fracture force of the tibia was decreased ($P<0.01$) when the low nPP diet was fed without phytase compared to the control diet (Table 3). The addition of either phytase source increased tibia fracture force compared to the low nPP diet and were similar results compared to the control diet. When cross sectional area of the tibia was considered to determine bone strength, there was only a trend ($P<0.086$) for the same response observed for fracture force. Tibia ash was decreased ($P<0.04$) when the low nPP diet was fed compared to the control diet. Neither phytase source resulted in significantly higher tibia ash compared to the low nPP diet, but Natuphos®

Table 1: Composition of the experimental diets

	5-8 wk			8-11 wk			11-14 wk			14-17 wk		
Ingredients	Control	Low P	WB[1]	Control	Low P	WB	Control	Low P	WB	Control	Low P	WB
Ground corn	54.62	56.15	51.95	60.68	62.25	58.03	62.70	64.24	60.00	66.30	67.85	63.60
Dehulled soybean meal	38.75	38.15	38.75	32.59	32.34	31.99	29.52	29.26	28.91	24.88	24.64	24.29
Wheat bran	0.00	0.00	3.27	0.00	0.00	3.27	0.00	0.00	3.27	0.00	0.00	3.27
Choice white grease	2.62	2.05	3.38	3.11	2.54	3.87	4.80	4.25	5.58	6.07	5.50	6.84
Dicalcium phosphate	1.90	1.36	1.32	1.63	1.08	1.04	1.33	0.79	0.75	1.21	0.66	0.63
Limestone	1.07	0.88	0.36	0.37	0.37	0.36	0.37	0.37	0.37	0.37	0.37	0.37
Sodium bicarbonate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-methionine	0.27	0.27	0.27	0.22	0.22	0.22	0.15	0.15	0.15	0.11	0.11	0.11
L-lysine-HCl	0.11	0.12	0.12	0.16	0.16	0.16	0.02	0.02	0.02	0.00	0.00	0.00
Vitamin premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Trace mineral premix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated nutrient levels												
Crude Protein, %	23.00	23.00	23.00	20.50	20.50	20.50	19.00	19.00	19.00	17.25	17.25	17.25
ME, kcal/kg	3,000	3,000	3,000	3,100	3,100	3,100	3,225	3,225	3,225	3,350	3,350	3,350
Calcium, %	0.95	0.75	0.75	0.80	0.60	0.60	0.72	0.52	0.52	0.66	0.46	0.46
Nonphytate P, %	0.50	0.40	0.40	0.42	0.32	0.32	0.38	0.28	0.28	0.35	0.25	0.25
Total P, %	0.75	0.65	0.67	0.68	0.58	0.59	0.61	0.51	0.52	0.57	0.47	0.48

[1]WB=wheat bran

[1]WB = wheat bran, [2]Vitamin premix provided per kg diet: vitamin A (all-trans-retinyl acetate), 11,000 IU; cholecalciferol, 5,000 ICU; vitamin E (all-rac-α-tocopherol acetate), 35 IU; menadione (as menadione sodium bisultite), 2.75 mg; riboflavin, 10 mg; Ca pantothenate, 20 mg; nicotinic acid, 80 mg; vitamin B_{12}, 0.025 mg; vitamin B_6, 4.3 mg; thiamin (as thiamin mononitrate), 2.9 mg; folic acid, 2.2 mg; biotin, 0.2 mg; vitamin C, 0.10 g; selenium, 0.275 mg; and ethoxyquin, 125 mg. [3]Mineral premix supplied per kg of diet: manganese, 100 mg; zinc, 100 mg; iron, 50 mg; copper, 10 mg; iodine, 1 mg.

Table 2: Effect of dietary phosphorus (P) concentration and phytase source on growth performance of commercial toms and resulting litter P concentration[1]

Treatment	Body Weight (kg)				Cumulative Feed Conversion (kg:kg)				Litter P		
	8 wk	11 wk	14 wk	17 wk	8 wk	11 wk	14 wk	17 wk	Total P	Soluble P	Soluble P
									---------- (% DM) ----------		(% of Total P)
Control	4.38[a,]	8.13[a]	11.99[a]	15.52[a]	1.84[b]	2.20[b]	2.54[b]	2.78[b]	1.267[a]	0.187[ab]	14.9[c]
Low P	4.23[b]	7.75[c]	11.51[b]	14.87[c]	1.93[a]	2.33[a]	2.66[a]	2.91[a]	0.988[b]	0.160[b]	16.7[bc]
Wheat bran	4.25[b]	7.87[bc]	11.59[b]	15.27[b]	1.91[a]	2.27[a]	2.61[a]	2.84[ab]	0.934[b]	0.177[ab]	19.3[ab]
Natuphos	4.26[b]	7.90[b]	11.61[b]	15.05[bc]	1.90[a]	2.29[a]	2.65[a]	2.88[a]	0.916[b]	0.197[a]	22.3[a]
SEM	0.03	0.04	0.07	0.08	0.01	0.02	0.02	0.03	0.072	0.01	1.2
ANOVA	---------- Probabilities ----------										
Treatment	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.012	0.006	0.046	0.002

[a-c] Means in a column with no common superscripts differ significantly ($P \leq 0.05$). [1]Means represent 8 pens per diet and 25 toms per pen.

Table 3: Effect of dietary phosphorus (P) concentration and phytase source on bone characteristics in commercial toms

Treatment	Fracture force (N) [1]			Bone Strength (MPa) [1]			Bone Ash (%)[1]			Serum Pyridinoline[2] (nmol/l)
	Femur	Tibia	Ulna	Femur	Tibia	Ulna	Femur	Tibia	Ulna	
Control	2004	760[a]	1762	7.79	93.8	15.9	52.5	57.3[a]	55.2	20.1
Low P	1943	638[b]	1681	7.52	80.7	16.7	51.7	55.9[b]	54.4	20.9
Wheat bran	2056	702[a]	1682	8.06	89.3	16.2	51.6	56.2[b]	54.9	21.0
Natuphos	2034	733[a]	1808	7.84	90.5	17.8	51.6	57.1[ab]	53.5	20.1
SEM	40	21	100	0.18	3.6	0.9	0.5	0.4	0.8	1.1
ANOVA	Probabilities									
Treatment	0.223	0.002	0.759	0.226	0.086	0.447	0.460	0.037	0.418	0.892

[a,b]Means in a column with no common superscripts differ significantly ($P \leq 0.05$). [1]Means represent 8 pens per diet and 3 toms per pen. [2]Means represent 8 pens per diet and 1 tom per pen.

fed birds had tibia ash values that were similar to tibia ash results for adequate nPP fed birds. No effects of treatment on femur or ulna fracture force, bone strength or ash or serum pyridinoline were noted.

Discussion

The 0.1 percentage unit reduction in dietary nPP in the low P diet compared to the control diet resulted in dietary P differences that ranged from about 20% in the 5-8 wk phase to 30% in the 14-17 wk phase with an average reduction of 25%. Litter P was also reduced by about 25% when the low nPP diet was fed with or without phytase. This observation confirmed that dietary nPP was reduced by about 25% when the low nPP diet was fed compared to the control (adequate nPP) diet. The control diet was formulated to provide nPP at NRC (1994) recommendations for 3-wk phases of feeding. The reduction in dietary nPP in the current study resulted in a reduction in body weight that was not observed by Atia *et al.* (2000) when the authors fed 73% of the NRC (1994) recommendations for turkeys on a 4-wk phase basis. Dietary calcium was maintained at NRC (1994) concentrations when 73% of the nPP requirement was fed in the report by Atia *et al.* (2000). The increase in feed conversion when the low phosphorus diet was fed with or without phytase was due to decreased body weight gain as feed intake was similar across treatments.

Roberson (2003) reported that feeding 75% of the NRC (1994) requirements for dietary calcium and nPP to toms consuming a mash diet decreased tibia and ulna fracture force at 11 wk of age. Tibia fracture force was also reduced at 14 wk of age when 75% of the NRC (1994) recommendations for calcium and nPP were fed compared to 105% of the NRC (1994) recommendations. Although there was a linear bone fracture force responses to dietary calcuim and nPP at 14 wk of age for the ulna, the effects of feeding either 75 or 105% of NRC (1994) recommendations for calcium and nPP were less pronounced. The results of the current study indicate that the tibia is the most reliable of the bones measured in the experiment to evaluate calcium and phosphorus status in growing turkeys. Roberson *et al.* (2004) also found the tibia to be the most sensitive bone to detect differences in bone fracture force when comparing low [below NRC (1994) recommendations] to medium [NRC (1994) recommendations] concentrations of dietary nPP. Significant ($P<0.05$) differences in tibia fracture force due to dietary calcium and nPP concentrations were not observed for bone strength measurements due to correction for cross sectional area. Hence, the size of the shaft of tibiae from birds fed the low nPP diet without phytase compared to the control birds was a factor in fracture force determination.

There were no significant effects of dietary treatment on femur fracture force or bone strength. Lilburn (1994) reported that the femur may be the weak link with respect to long bone development abnormalities in turkeys. Field reports in the U.S. have identified the femur as the bone most likely to spontaneously fracture in heavy toms. Although the femur does break in a shattering response compared to a clean break when other bones are sheared, high variability in femur fracture responses make this bone less sensitive to dietary calcium and nPP treatments (Roberson, 2003; Roberson *et al.*, 2004; Klunzinger *et al.*, 2005).

The bone ash data also indicate that the tibia was the most useful bone to evaluate dietary treatments in this study. This agrees with a previous study in our laboratory when tom turkeys were grown to 17 wk of age (Roberson *et al.*, 2004). Although ulna ash has been shown to be a sensitive indicator of dietary calcium and nPP status in 15-wk-old toms (Roberson *et al.*, 2004), this characteristic has not been useful at later ages (Roberson *et al.*, 2004; Klunzinger *et al.*, 2005).

Serum pyridinoline has previously been reported to be higher in 17-wk-old toms when a low nPP diet was fed (Roberson *et al.*, 2002). However, the response throughout the growing turkey trial was inconsistent. Hoshino *et al.* (1998) showed that pyridinoline is

increased in the urine of rats fed a P deficient diet that induced a rachitic status in the animals. Withdrawal of vitamins, trace minerals and inorganic P supplements from a finishing pig diet for 27 d increased serum pyridinoline and coincided with decreased metacarpal ash weight and concentration (Shaw, 2001). Serum pyridinoline appears to be an unreliable predictor of P status in market age turkeys when dietary nPP levels differ by 0.1 percentage units and are fed close the NRC (1994) recommendations.

The 0.04%-unit increase in soluble P in litter when Natuphos® was fed compared to the low nPP diet is an environmental concern that was reported by DeLaune *et al.* (2001) in a rainfall simulation study for runoff from test plots in which litter from broilers fed phytase. McNaughton and Barnes (2004) reported that dried excreta samples from broilers fed phytase had higher soluble P content. Although soluble P concentration in the litter was not significantly increased by phytase over the control diet in this study, the increase in the proportion of soluble P to total P still poses a perception problem with phytase use in animal diets from the public. Applegate *et al.* (2003) reported that neither soluble P content nor soluble P as a percent of total P is increased in broiler litter when phytase is fed as long as the dietary nPP level is below the requirement for the bird. Although dietary nPP was formulated to be below NRC (1994) requirements for nPP in the low nPP diet in our study, the growth performance data indicate that the toms could have possibly been fed less P especially in the finisher (14-17 wk) phase. The reductions in body weight throughout the trial appear to be due to growth depressing effects early in growth which carried over to market age. Further work by Angel *et al.* (2005) on the issue of dietary phytase on water-soluble phosphorus in excreta showed there was no increase in excreted water-soluble phosphorus when phytase was fed to broilers, turkeys or pigs. The authors reported that increases in water-soluble phosphorus levels of excreta were a function of microbial activity inherently present in excreta rather than due to phytase addition to the diet. As litter moisture at 17 wk was 52%, differences in the proportion of P that was water soluble may be due in part to inherent microbial activity in the litter.

The results show that phytase activity from wheat bran was just as effective as a commercial source to improve bone integrity when toms are fed a low nPP diet. The price of the commercial source of phytase used in the study was $1.76 (U.S.)/kg. The use of wheat bran as the phytase source resulted in a reduction of an average of 10 cents (U.S.)/ton of feed over all the phases (higher in later phases). However, plant sources of phytase may be more variable within source (Eeckout and de Paepe, 1994) and are generally considered to be a less desirable source of phytase activity than purified commercial sources. Wheat bran phytase is a viable option to be used as at least part of the phytase source in turkey diets and can result in increased profits to the grower and/or company producing turkey meat. Further work is needed to evaluate the dietary P needs of toms approaching market age to prevent an increase in water soluble P content of the litter.

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[2]Pillsbury, General Mills Sales, Inc., Minneapolis, MN

[3]Natuphos 600®, BASF, Parsippany, NJ

[4]Instron Universal Testing Machine Model 4202, Canton, MA