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The effects of phytase supplementation and dietary phosphorus level on performance and on tibia ash and phosphorus contents in broilers fed maize-soya-based diets

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ABSTRACT

To test phytase efficiency, 4 dietary treatments including a positive control (T1), negative control (T2, containing 0.10% less total phosphorus than T1), negative control plus 500 FTU phytase (T3), and low-negative control plus 500 FTU phytase (T4, containing 0.13% less total phosphorus than T1) were used. Reducing the available phosphorus level of the broiler diet from 0.47% to 0.37% during the starter, and from 0.37% to 0.26% during the grower-finisher period significantly ($P<0.01$) depressed growth performance as compared with T1. Phytase supplementation significantly ($P<0.01$) improved the growth performance of the birds for both starter and grower periods. Tibia ash and phosphorus content in both the T3 and T4 groups were similar to T1 and higher ($P<0.01$) than in T2. Phytase supplementation of both negative control diets significantly ($P<0.01$) reduced the phosphorus level in excreta. It can be concluded that with phytase supplementation, the total phosphorus level in broiler diets can be decreased by 0.13%.

KEY WORDS: broiler, phytase, growth performance, tibia ash, phosphorus

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INTRODUCTION

A significant portion of the phosphorus (P) in mature cereal grains and oilseeds is present as phytate P in mixed salts of phytic acid (myoinositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate); phytic acid is a ubiquitous component of plant-sourced feed ingredients which encompasses approximately two-thirds of total plant P (Hughes et al., 2009; Woyengo et al., 2010). Phytate P is utilized with an availability from 0% to 50% in poultry, depending on age and metabolic adaptation in critical circumstances, hence, expensive inorganic P sources are routinely added to poultry diets to meet P requirements; this practice, however, ultimately, leads to a large portion of dietary P not being utilized by the animal but being excreted in faeces (Hughes et al., 2008; Woyengo et al., 2010). Several alternative methods have been devised over recent years in order to reduce the negative impact of phytate P on the environment and poultry performance. The use of one of these strategies, which includes the administration of microbial phytase, has increased remarkably since the early 1990s and has become a standard practice (Ceylan et al., 2003; Francesch and Geraert, 2009).

It must be mentioned that different phytases have various characteristics depending on the source from which they are derived, so they do not have the same effect and activity in the digestive tract. Therefore, each phytase preparation for poultry must be tested *in vivo* to ensure its efficacy (Onyango et al., 2005b; Hughes et al., 2008).

The current study was designed to evaluate the effect of microbial phytase supplementation on growth performance, tibia ash and P contents in tibia bones and in excreta of broilers.

MATERIAL AND METHODS

Animals and management

The research was carried out in a poultry house of the Animal Science Department of Ankara University. Two hundred and forty Ross 308 one-day-old male broiler chicks were randomly allocated to four dietary treatments, each with six replicates of ten birds placed in cage pens (90 × 85 cm). The birds had a similar initial mean body weight of 40 ± 0.1 g. On the first day the ambient temperature was 33°C, which was gradually decreased to 24°C at 3 weeks of age, maintained using a thermostatically controlled heater fan. The relative humidity of the house during the trail was 50% ± 5%. The house was artificially ventilated and during the trail all of the birds were kept under 24 h lighting regimens provided by incandescent lights. Each cage was equipped with automatic nipple drinkers, feeders were

adjusted outside of the cage. Water and experimental diets (in mash form) were offered *ad libitum*. All birds were vaccinated against Gumboro disease on the 14th day. The experimental design regarding the dietary treatments for starter and grower-finisher periods is shown in Table 1. The starter and grower-finisher diets were based on maize-soyabean meal and were offered to the birds from 0-21 and 21-40 days of age, respectively (Table 2). All diets were formulated to meet or exceed NRC (1994) requirements.

Table 1. Phytase added and available P level in experimental diets

Treatments	Phytase activity added, FTU/kg	Dietary P level, % ¹	
		starter diet	grower-finisher diet
T1-positive control	0	0.70 (0.47)	0.612 (0.37)
T2-negative control (NC)	0	0.607 (0.37)	0.507 (0.26)
T3-NC plus phytase	500	0.609 (0.37)	0.504 (0.26)
T4-low NC plus phytase	500	0.57 (0.34)	0.468 (0.23)

¹ parenthesis indicates available P level and calculated according to Selle and Ravindran (2007)

Table 2. Composition of starter and grower-finisher diets, g/kg air-dry matter

Indices	Dietary treatments ¹							
	T1	T2	T3	T4	T1	T2	T3	T4
	starter				grower - finisher			
<i>Ingredients</i>								
maize	497.7	507	507	510.5	526.6	536.4	536.4	538.6
soyabean meal	384	383	383	381	371	370	370	369
fish meal	40	40	40	40				
sunflower oil	45.2	42	42	41.3	66.5	63	63	62.5
limestone	13.2	13.7	13.7	14.6	15.25	16	16	17
dicalcium phosphate	9.3	3.7	3.7	2.05	11.45	0.54	0.54	0.37
NaCl	4	4	4	4	4.15	4.15	4.15	4.15
L-lysine %	0.6	0.6	0.6	0.6	0.25	0.25	0.25	0.25
DL-methionine %	2.5	2.5	2.5	2.45	2.3	2.3	2.3	2.3
vitamin-mineral premix ²	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
anticoagulant ³	1	1	1	1				
phytase preparation ⁴			0.1	0.1			0.1	0.1
ME, MJ/kg (calculated)	12.83	12.83	12.83	12.83	13.39	13.39	13.39	13.39
<i>Analysed</i>								
dry matter, %	90.08	89.84	89.36	90.16	90.20	90.12	90.30	89.74
crude protein, %	23.38	23.06	23.16	22.83	20.63	19.86	19.43	19.64
crude fat, %	7.91	7.58	7.89	7.93	9.51	9.69	9.38	9.22
crude ash, %	6.30	6.01	6.01	5.90	6.14	5.73	5.72	5.77
total P, %	0.70	0.607	0.609	0.57	0.612	0.507	0.504	0.468
phytase activity, FTU	-	-	540	550	-	-	560	730

¹ see Table 1; ² provides per kg of diet: mg: vit. A (all-*trans*-retinol) 3.6, vit. D (cholecalciferol) 0.038, vit. E 50, vit. K₃, 5, vit. B₁, 3, vit. B₂, 6, vit. B₆, 5, vit. B₁₂, 0.030, niacin 25, Ca-D-pantothenate 12, folic acid 1, D-biotin 0.05, choline chloride 400, apo-carotenoid acid ethyl ester 2.5, Mn 80, Fe 30, Zn 60, Cu 5, I 2, Co 0.5, Se 0.15; ³ CYGRO®; ⁴ 5000 FTU/g, see Material and Methods

Analysis

Hostazym®P phytase, supplied by Huvepharma AD, was used in the experimental diet. This preparation contains 5000 FTU/g according to the producer's declaration. The manufacturer recommends adding it to broiler diets at a rate of 100 g/ton.

Mortality was recorded daily, feed intake (FI) and body weight were recorded weekly. FI data in replications were corrected by withdrawing the calculated consumption of the dead bird. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated for the periods of day 1-21 and 22-40. During the trial, birds that could not stand up because of twisted legs were recorded for incidence of leg problems. At the end of the experiment, 2 chickens per pen with a weight near the average were selected and killed by cervical dislocation for processing. The birds were weighed, plucked, and eviscerated. Carcass, drumsticks (with bones), breast meat (with bones), and abdominal fat were excised and weighed, then calculated as a percent of live body weight. The left tibia from each bird was excised, sealed in plastic bags and stored at -20°C until further analysis.

Raw materials and diets were analysed for nutrient contents according to AOAC (2005). Phytase activity in diets was measured according to Gizzi et al. (2008). A unit of phytase activity was defined as the amount of inorganic phosphate released from myoinositol hexakisphosphate after 30 min incubation at 37°C with 100 µl of extract (obtained by extracting 50 g of feed with 500 ml distilled water) in 300 µl acetate buffer (0.25 M; pH 5.5).

Before analysis, meat and fat were gently removed from tibia bones. The bones were dried overnight at 100°C, extracted in ether for 6 h, and burnt to ash in a muffle furnace at 600°C. The ash from each tibia was used for phosphorus analysis according to AOAC (2005). On the last day of the experiment, approximately 50 g excreta samples from three replicates per group were collected, dried, and analysed for phosphorus content.

Statistical analysis

The data were analysed as a completely randomized block design with 4 dietary treatments and 6 replicates using the ANOVA procedure of the SAS (1996). All percentage data were subjected to arcsine square root transformation. When significant differences among groups were found, means were separated using the Tukey HSD test.

RESULTS AND DISCUSSION

The performance results of the experiment are given in Table 3. Reducing the dietary calculated available phosphorus (aP) level of the broiler diet from 0.47% to 0.37% during the starter period and from 0.37% to 0.26% during the grower-finisher period significantly ($P<0.01$) decreased BWG, FI, and FCR as compared with the positive control (T1). The lowered body weight was due to the P deficiency in broilers fed at the 0.37% and 0.26% aP level, which is slightly below the recommended level for broilers during the starter and finisher periods, respectively (NRC, 1994). Some previous researchers have also reported poor performance of broilers fed diets with a P deficiency (Sohail and Roland, 1999; Bozkurt et al., 2006). The growth performance of T1 broilers during the first 21 d of age was approximately similar to what has been shown by Onyango et al. (2005a), Olukosi et al. (2007), and Woyengo et al. (2010) for chickens receiving adequate nutrients in their diets. Phytase supplementation significantly ($P<0.01$) improved growth performance of the birds and the best ($P<0.001$) FCR was obtained with the broilers fed diets T3 and T4.

Table 3. Effects of phytase supplementation on feed intake (FI) and feed conversion ratio (FCR) of broiler

Treatments ¹	BWG ² , g/day			FI, g/period			FCR, kg feed /kg BWG		
	1-21	22-40	1-40	1-21	22-40	1-40	1-21	22-40	1-40
T1	31.9 ^a	70.4 ^{ac}	50.2 ^a	0.840 ^a	2.17 ^a	3.01 ^a	1.25 ^a	1.62	1.49 ^a
T2	26.4 ^b	52.1 ^b	38.6 ^b	0.735 ^b	1.66 ^b	2.40 ^b	1.32 ^b	1.68	1.55 ^b
T3	34.8 ^c	74.3 ^c	53.6 ^a	0.904 ^c	2.30 ^a	3.20 ^a	1.24 ^a	1.63	1.49 ^a
T4	31.6 ^a	75.8 ^c	52.6 ^a	0.832 ^a	2.27 ^a	3.11 ^a	1.25 ^a	1.58	1.47 ^a
SEM ³	0.5	2.1	1.1	0.09	0.05	0.06	0.01	0.02	0.01
P	0.01	0.001	0.02	0.0	0.0	0.0	0.004	0.1	0.03

¹ see Table 1; ^{a-c} means followed by different letters within columns are significantly different ($P<0.05$); ² BWG - body weight gain; ³ SEM - standard error of mean

The release of energy and amino acids by phytase is a disputable issue. Some research suggests up to 2% improvement in AME_N values and amino acid digestibility, more conservative estimates are 0.062 MJ ME/kg, with no increase in amino acid availability (Leeson and Summers, 2005). In the present study, the better growth of broilers offered feeds containing phytase over the positive control birds can be attributed to the activity of the phytase used in the experiment. There were no significant differences, however, between T1 and T3 regarding FCR; the BWG of T3, which exceeded 5%, was higher than in T1. Bozkurt et al. (2006) reported that the growth rate and FCR of broilers fed low-P diets containing microbial phytase are comparable with or even better than those obtained in broilers fed the standard P diets. Many researchers have observed an improvement

due to dietary phytase supplementation in BWG and FI during the first 21 d of age (Denbow et al., 1995; Cabahug et al., 1999), whereas others reported no effect (Boling-Frankenbach et al., 2001). These contrasting results may be due to a number of factors, including phytase source (type, source, phytate content), and dietary characteristics (processing, vitamin D₃ level, Ca:P ratio) (Ravindran et al., 1995).

As can be seen from Table 4, lowering the total P level of the diet by 0.13% to below the P requirement recommended by NRC (1994) resulted in significantly ($P<0.05$) higher mortality than in the positive control group. Phytase supplementation decreased the mortality and there were no significant differences among the positive control and phytase-supplemented groups. Mortality results in the current experiment confirm the findings of previous reports (Shirley and Edwards, 2003; Persia and Saylor, 2006; Jiang et al., 2011). Walk (2006) showed that the mortality rate in 42-day old male broilers was 24% in birds fed the negative control diet, which contained 0.26% less total P than recommended by NRC (1994), and 6% in birds fed 500 FTU/kg phytase-supplemented diets ($P<0.05$).

Table 4. Effects of phytase supplementation on mortality, bone development, excreta phosphorus

Treatments ¹	Abnormal legs %	Mortality %	Tibia ash % of DM	Tibia P % of dry-defatted tibia	Excreta P % in air dried excreta
T1	1.8 ^a	3.3 ^a	41.7	8.3 ^a	1.5 ^a
T2	16.6 ^b	20.0 ^b	38.4	6.8 ^b	1.2 ^b
T3	3.7 ^a	11.6 ^{ab}	41.5	8.4 ^a	1.2 ^b
T4	1.8 ^a	11.6 ^{ab}	42.9	8.9 ^a	1.0 ^c
SEM ²	2.7	3.6	1.3	0.3	0.01
P	0.03	0.05	0.4	0.006	0.0001

¹ see Table 1; ^{a-c} values followed by different letters within columns are significantly different ($P<0.05$); ²SEM - standard error of mean

Leg problems were also affected by dietary treatments ($P<0.05$). Broiler chickens receiving the P-deficient diet (T2) had a higher incidence of abnormal legs in comparison with the remaining groups ($P<0.05$; Table 4).

In the present study, supplemental phytase did not influence tibia ash, which is in accordance with the results of Perney et al. (1993) and Pintar et al. (2004), but in contrast with those of Sohail and Roland (1999) and Woyengo et al. (2010) from maize-soyabean based diets.

In the T3 and T4 groups, as in the T1 group, tibia P content was higher than in T2 ($P<0.01$), so presumably the added phytase liberated P from phytic acid molecules to satisfy the broilers' requirement for deposition of P in bones. This result is consistent with previous reports that phytase supplementation results in improved bone strength and bone mineralization in broilers fed low-P diets (Dilger et al., 2004; Woyengo et al., 2010).

The content of P in broiler excreta at 40 d of age in group T4 was lower than in group T1 ($P < 0.01$; Table 4). This result corroborates the findings of Waldroup et al. (2000) and Yan et al. (2000), who reported the reduction of excreta P output by approximately 25% to 28%, respectively, when broiler diets were supplemented with phytase. Reported values for P level in broiler excreta vary widely, and can average even 2.1%, with a range of 1.3% to 3.4% (Waldroup et al., 2000).

As shown in Table 5, no statistical differences were observed for carcass yields or individual part yields between treatments. Pillai et al. (2006) showed that in broilers fed diets with an adequate level of Ca and deficient level of P during the starter and grower periods, microbial phytase supplementation prevented the negative effects of P deficiency on carcass and breast yields. Also Scheideler and Ferket (2000) reported the lack of significant effects of exogenous phytase on broiler carcass yields.

Table 5. Effects of phytase supplementation on some carcass characteristics, % of live body weight

Treatment ¹	Carcass yield	Drumsticks	Breast meat	Abdominal fat
T1	68.9	29.0	33.1	0.9
T2	67.7	30.1	33.3	0.8
T3	70.1	28.8	33.1	1.1
T4	71.2	28.4	34.3	1.3
SEM ²	0.8	0.5	0.6	0.1
P	0.1	0.1	0.5	0.5

¹see Table 1; ²SEM - standard error of mean

CONCLUSIONS

It can be concluded that under the conditions used in the current study, phytase supplementation permits reducing the total P level of the broiler diets by up to 0.13% without any adverse effects on broiler growth and bone development. The cost benefits and lower pollution of the environment by broiler production are additional values of phytase supplementation.

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