Journal of Animal and Veterinary Advances 6 (12): 1434-1442, 2007
ISSN: 1680-5593

Effects of Dietary Phytase, Calcium and Phosphorus on Performance, Nutrient Utilization and Blood Parameters of Male Broiler Chickens

[1]A. Hassanabadi, [1]A. Alizadeh-Ghamsari and [2]M.A. Leslie
[1]Department of Animal Science, Faculty of Agriculture, University of Zanjan,
P.O. Box 313, Zanjan, Iran
[2]Masterfeeds Inc., 1020 Hargrieve Road, London, Ontario, Canada, N6E 1P5

Abstract: An experiment was conducted to evaluate the effects of microbial phytase and dietary Calcium (Ca) and phosphorus levels on productive traits, tibia ash percentage, apparent retention of Ca, tP, N and also on number of blood factors in male broiler chickens. The experiment was a 2×3 factorial with a completely randomized design. Each treatment consisted of 5 replicates of 12 male broilers, for a total of 360 chicks. Broiler chickens were fed two dietary levels of Ca and nPP (NRC, 1994 recommended levels and 80% of its recommendation levels) and three levels of phytase (0, 300 and 600 FTU kg^{-1} of diet) from 7-42 days of age. Experimental diets were formulated to be similar nutrients except for Ca and tP. During the experiment Body Weight (BW), Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) were measured weekly. Tibia ash percentage and blood serum Ca, P and Alkaline Phosphatase (ALP) were measured at 28 days of age. Nutrient apparent retention was measured at 42 day. Phytase addition increased ($p<0.05$) BW (21 and 42 day) and ADG during 7-21 and 7-42 days of age. FCR also was improved ($p<0.05$) during 7-21 and 7-42 day by phytase addition. Neither phytase addition nor Ca and nPP levels had significant effects ($p>0.05$) on ADFI during the periods of the experiment. Phytase addition decreased ($p<0.05$) serum ALP linearly and also decreased feed cost/kg BW yield. Low dietary Ca and nPP levels improved BW (21 day), ADG and FCR during 7-21 day ($p<0.05$). Phytase addition to low Ca and nPP diets was more efficient ($p<0.05$) than normal Ca and nPP diets on BW, ADG, ADFI, FCR, serum ALP concentration and Ca, tP and N retention. Phytase supplementation increased ($p<0.05$) serum P concentration, tibia ash percentage and Ca, tP and N retention. Low dietary Ca and nPP levels, increased ($p<0.05$) serum ALP and Ca, tP and N retention and decreased ($p<0.05$) serum P concentration. These data indicate that phytase improves BW, ADG, FCR and decreases serum ALP and feed cost/kg BW yield of broiler chickens. Low dietary Ca and nPP levels, regardless to phytase levels, improve BW, ADG and FCR. Phytase addition to low Ca and nPP diets is more efficient than normal Ca and nPP diets on performance and Ca, tP and N retention.

Key words: Broiler, performance, phytase, nutrient utilization, blood parameters

INTRODUCTION

About two-thirds of the total P contained in feed ingredients of plant origin occurs as phytate (Nelson, 1967). Because corn and soybean meal make up a substantial portion of diets for chickens, the availability of P in feedstuffs of plant origin is generally very low (Harland and Oberleas, 1999; Ravindran *et al*., 1999). Bioavailability estimates of P in corn and soybean meal for pigs and poultry range from 10-30% (Nelson, 1967; Jongbloed and Kemme, 1990). This low availability of phytate P poses two problems for producers: the need to add inorganic P supplements to diets and the excretion of large amounts of P in manure. In addition to low P availability, phytate limits availability of several other essential nutrients. Formation of insoluble complexes between phytate, calcium and other cations render several nutrients unavailable. Phytic acid has chelating potential and forms a wide variety of insoluble salts with di- and trivalent cations at neutral pH (Harland and Oberleas, 1999). Phytase is an enzyme that hydrolyzes the release of P from the phytate molecule (Kies, 1999). It has been shown to be effective when the Ca and nonphytate P (nPP) concentration of the diet is reduced, thus reducing the need for inorganic P addition (Denbow *et al*., 1995; Gordon and Roland, 1998; Sohail and Roland, 1999;

Corresponding Author: A. Hassanabadi, Department of Animal Science, Faculty of Agriculture, University of Zanjan, P.O. Box 313, Zanjan, Iran

Yan *et al*., 2001). However, the effect of phytase in nutritionally adequate Ca and nPP diets has been studied to a much lesser extent and with varied results. Some studies have shown a positive effect or an extraphosphoric effect of phytase (Cabahug *et al*., 1999; Keshavarz, 2000; Waldroup *et al*., 2000; Watson *et al*., 2005) whereas other studies did not see an improvement by phytase in nutritionally adequate diets (Gordon and Roland, 1997; Sebastian *et al*., 1997).

Phytate P is either unavailable or poorly utilized by monogastric animals due to insufficient quantities of endogenous phytase (Nelson, 1967). Phytate complexes with minerals and precipitates at the pH of the intestine (Harland and Oberleas, 1999) and makes these minerals unavailable for intestinal absorption. Phytase (EC.3.1.3.8), *myo*-inositol hexakisphosphate phosphohydrolase, is the enzyme that releases P from phytate molecule (Harland and Oberleas, 1999). The efficacy of microbial phytase to improve dietary P bioavailability has been reported by several researchers (Simons *et al*., 1990; Denbow *et al*., 1995; Ravindran *et al*., 1995; Kornegay *et al*., 1996; Mitchell and Edwards, 1996; Gordon and Roland, 1997).

The objective of this research was to evaluate the effects of phytase on growth performance, tibia ash percentage and apparent retention of calcium, total phosphorus and N and on number of blood factors in male broiler chickens in nutritionally normal Ca and nPP diets as well as low Ca and nPP diets for broiler chickens.

MATERIALS AND METHODS

A total of 360 male broiler chickens from Arbor Acres plus (AA+) strain were allotted to 6 dietary treatments in completely randomized design. Each treatment was replicated 5 times with 12 chicks per replicate. Commercial brooding and management procedures were followed and all chicks were fed a typical commercial broiler starter ration for the first 6 days. On 7 day, after an overnight fast, the chicks were weighed, wing-banded and allotted to treatments. They were then fed the experimental diets (Table 1) through to 42 day. Chicks were housed in environmentally controlled floor pens (1.5×1.5 m). Chicks, feed and water were checked twice daily. Feed and water were provided on an ad libitum basis throughout the experiment. Body weight and feed consumption were recorded on a pen basis at weekly intervals. Corn-soybean meal diets adequate in all nutrients (except Ca and nPP for experimental purposes) were used. Experimental diets were formulated to be isoenergetic and isonitrogenous and the diets met or exceeded all other nutrient requirements (NRC, 1994). The dietary treatments for 7-21 day were: C-SBM, 1.0% Ca and 0.45% nPP (normal Ca and P) and C-SBM, 0.80% Ca and 0.36% nPP (low Ca and nPP); each of these diets were supplemented with 0, 300 and 600 phytase (Natuphos 10000; BASF Corp., Germany) units/kg of diet to make up 6 dietary treatments. The dietary treatments for 21-42 day were: C-SBM, 0.93% Ca and 0.35% nPP (normal Ca and P) and C-SBM, 0.71% Ca and 0.28% nPP (low Ca and nPP); each of these diets were supplemented with 0, 300 and 600 phytase units/kg of diet to make up 6 dietary treatments for 21-42 days of age. The three experimental diets in each experimental period were made by adding sand to the reduced Ca and nPP diets in place of the dicalcium phosphate. At 28 day, one bird close to the pen body weight mean was selected from each pen, blood samples were obtained by cardiac puncture for subsequent determination of Ca, P and ALP. The bird was then killed and the left tibia was carefully removed and was frozen until analyzed. The bones were extracted for 48 h with ethyl alcohol followed by a 48-h extraction in ethyl ether. They were then dried for 24 h at 100°C and the dry fat-free bones ashed in a muffle furnace overnight at 600°C. Ash weight was calculated as a percentage of dry fat free bone weight. For determination of Ca, total P and N retention at 42 day of age, clean stainless steel collection trays were placed under each cage (3 per treatment) and the selected birds were located individually into cages to collect the excreta. Excreta from the birds totally were collected for 72 h. A subsample of excreta was collected in polyethylene bags, weighed and dried. Excreta were mixed thoroughly, frozen at -20°C and oven-dried. Prior to chemical analysis, these samples were ground (0.5 mm, screen). Diets and excreta samples were analyzed for Ca and tP by Atomic Absorption Spectrophotometer and N by Kjeltec Auto Analyzer 1030/Dijestion System 20 (AOAC, 2003). Serum samples were analyzed for Ca, tP and alkaline phosphatase by commercial kits. At 42 day one bird close to pen body weight mean was killed and the edible carcass weight was determined. Mortality rate was recorded daily through the experiment.

Statistical analysis: Data were analyzed as completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The dietary treatments were arranged as a factorial and contrasts were used to determine main effects of phytase and Ca and nPP and the interaction between phytase and Ca and nPP levels. The pen of chicks served as the experimental unit. Means were compared using Duncan's new multiple range test (Steel and Torrie, 1980). The level of significance was reported at $p<0.05$.

Table 1: Ingredients and nutrient composition of experimental diets

Diet	Starter (7-21 day)		Grower (21-42 day)	
Ca and P levels	Normal Ca and P	Low Ca and nPP	Normal Ca and P	Low Ca and nPP
Ingredient (%)				
Corn	54	54	55.84	55.84
Soybean meal	35.4	35.4	31	31
Fish meal (Anchovy)	3.5	3.5	4	4
Soybean oil	2.8	2.8	5.6	5.6
Dicalcium phosphate	1.65	1.15	1.18	0.65
Oyster shell	1.03	0.8	1.02	0.8
DL-Methionine	0.12	0.12	0.01	0.01
Common salt	0.3	0.3	0.25	0.25
Sodium bicarbonate	0.1	0.1	0.05	0.05
Sand	-	0.73	-	0.7
Mineral mix[1]	0.55	0.55	0.55	0.55
Vitamins mix[2]	0.55	0.55	0.55	0.55
Calculated composition				
ME (kcal kg^{-1})	2935	2935	3158	3158
CP (%)	21.11	21.11	19.72	19.72
Ca (%)	1.0	0.80	0.90	0.72
Total P (%)	0.77	0.61	0.59	0.44
nPP (%)	0.45	0.36	0.35	0.28
Ca:nPP	2.31	2.27	2.65	2.53
Lysine (%)	1.1	1.1	1	1
Methionine + Cystine (%)	0.9	0.9	0.71	0.71

[1]Mineral mix supplied the following per kg of diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4; Zn, 169.4 mg. [3]Vitamins mix supplied the following per kg of diet: Vitamin A, 18,000 IU; vitamin D3, 4,000 IU; vitamin E, 36mg; vitamin K_3, 4 mg; vitamin B_{12}, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg

RESULTS

The effects of phytase supplementation and Ca and nPP levels on growth performance of the chickens are summarized in Table 2 and 3.

Average daily feed intake: The main effects data indicated that the decreases in Ca and nPP levels and phytase supplementation in the diet numerically increased Average Daily Feed Intake (ADFI) in all of experimental periods (Table 2). Low Ca and nPP level diet supplemented with 600 FTU phytase resulted significantly more ADFI than normal Ca and nPP diet supplemented with 300 FTU phytase during 7-21 day. Furthermore, ADFI numerically was increased as the level of phytase was increased in both chicks fed normal and low Ca and nPP levels (Table 3).

Average daily weight gain and body weight: Phytase increased ($p<0.05$) ADG of chicks during the period of 7-21 and 7-42 day (Table 2). During these periods there was not significant difference between effects of 300 and 600 FTU phytase. The effect of these phytase levels in compare to 0 FTU phytase on ADG was significantly different. Low Ca and nPP levels also significantly ($p<0.05$) increased ADG of chicks when compared with normal Ca and nPP during 7-21 day. Phytase levels had no significant effect on ADG during the period of 21-42 day but during the entire experimental period (7-42 day) ADG was increased significantly ($p<0.05$) by 300 and 600 FTU phytase. Ca and nPP levels had no significant effect during the periods of 21-42 and 7-42 days. However, low Ca and nPP levels numerically increased the ADG at these periods. As shown in Table 3, ADG was increased as the level of phytase was increased in both chicks fed normal and low Ca and nPP levels. Live body weight of the chicks was significantly ($p<0.05$) increased by phytase at both 21 and 42 days of age. Low Ca and nPP diets significantly increased BW at 21 day but not at 42 day in compare to normal Ca and npp levels (Table 2). BW of the birds at day 42 of the experiment in chicks fed with both levels of Ca and nPP was increased as the level of phytase was increased (Table 2).

Feed conversion ratio: Feed Conversion Ratio (FCR) of the chicks was significantly ($p<0.05$) improved by phytase supplementation during both 7-21 and 7-42 days of experimental periods. There was not significant difference between 300 and 600 FTU phytase on FCR during these periods (Table 2). However, phytase effect on FCR was not significant during 21-42 day. Low Ca and nPP level diet significantly improved FCR of the chicks during 7-21 day but not at 21-42 and/or 7-42 day in compare to normal Ca and nPP level diet (Table 2). As shown in Table 3, FCR was improved as the level of phytase was increased in both chicks fed normal and low Ca and nPP levels and there was not significant difference between 300 and 600 FTU phytase.

Table 2: Effects of microbial phytase and dietary Ca and nPP levels on performance of broiler chickens

	BW (g)		ADG (g)			ADFI (g/b/d)			FCR (g/g)		
Treatment	21 day	42 day	7-21 day	21-42 day	7-42 day	7-21 day	21-42 day	7-42 day	7-21 day	21-42 day	7-42 day
Phytase (FTU/kg diet)											
0	595.9^{b}	2168.6^{b}	33.6^{b}	75.5	58.2^{b}	57.1	137.2	104.0	1.71^{a}	1.82	1.79^{a}
300	632.7^{a}	2304.8^{a}	36.0^{a}	80.0	62.2^{a}	56.8	140.9	105.8	1.58^{b}	1.76	1.72^{b}
600	648.3^{a}	2333.1^{a}	37.5^{a}	80.3	62.4^{a}	58.2	142.1	107.3	1.55^{b}	1.78	1.71^{b}
SE	9.20	35.67	0.56	1.73	1.11	0.68	1.75	1.11	0.02	0.02	0.02
Ca and P levels											
Normal Ca and P	01.0^{b}	2230.8	34.1^{b}	77.7	59.6	56.6	138.6	104.5	1.67^{a}	1.79	1.76
Low Ca and nPP	650.3^{a}	2306.9	37.3^{a}	79.5	62.2	58.1	141.4	106.8	1.56^{b}	1.78	1.72
SE	7.51	29.12	0.46	1.41	0.91	0.56	1.43	0.91	0.02	0.02	0.01

$^{a-b}$Means within each column with no common superscript differ significantly ($p<0.05$). ^{SE}Standard Error

Table 3: Interaction between microbial phytase and dietary Ca and nPP levels on performance of broiler chickens

		BW (g)		ADG (g)			ADFI (g/day)			FCR (g/g)		
Phytase	(FTU/kg											
Treatment	diet)	21 day	42 day	7-21 day	21-42 day	7-42 day	7-21 day	21-42 day	7-42 day	7-21 day	21-42 day	7-42day
Normal Ca and P	0	560^{c}	2114^{b}	31.4^{d}	74.1^{b}	56.4^{b}	56.7^{ab}	135.5	102.9	1.81^{a}	1.83	1.82^{a}
	300	601^{b}	2250^{ab}	34.6^{c}	78.0^{ab}	59.8^{ab}	55.8^{b}	138.1	103.7	1.62^{b}	1.77	1.73^{b}
	600	634^{ab}	2327^{a}	36.4^{abc}	81.1^{ab}	62.6^{a}	57.4^{ab}	142.3	107.1	1.58^{b}	1.76	1.71^{b}
Low Ca and P	0	632^{ab}	2222^{ab}	35.9^{bc}	76.1^{ab}	59.9^{ab}	57.5^{ab}	138.8	105.2	1.61^{b}	1.81	1.76^{ab}
	300	656^{a}	2339^{a}	37.4^{ab}	82.7^{a}	62.2^{a}	50.9^{ab}	143.6	107.5	1.55^{b}	1.74	1.73^{b}
	600	622^{a}	2359^{a}	38.5^{a}	78.9^{ab}	64.5^{a}	59.0^{a}	141.9	107.9	1.53^{b}	1.79	1.71^{b}
SE		13.01	50.45	0.79	45.2	1.57	0.97	2.47	1.57	0.03	0.03	0.02

$^{a-d}$Means within each column with no common superscript differ significantly ($p<0.05$). ^{SE}Standard Error

Tibia ash: Tibia ash percentage also was increased ($p<0.05$) by 600 FTU phytase in compare to chicks fed both 0 and 300 FTU phytase. The low Ca and nPP level diet had no significant effect on this characteristic (Table 4). As shown in Table 5, tibia ash was increased as the level of phytase was increased in both chicks fed normal and low Ca and nPP levels. There was not significant difference between 300 and 600 FTU phytase. However, to increase tibia ash percentage, 600 FTU phytase was more effective than 300 FTU (Table 5).

Calcium, total phosphorus and nitrogen apparent retention: Apparent retention of Ca, tP and N were increased by dietary phytase and also by low Ca and nPP level diet. The effect of dietary phytase on Ca and tP apparent retention was linear, but there was no significant ($p<0.05$) difference between 300 and 600 FTU phytase (Table 6). As shown in Table 7, retention of Ca, tP and N apparent retention were increased as the level of phytase was increased in both chicks fed normal and low dietary Ca and nPP levels.

Serum calcium, phosphorous and Alkaline Phosphatase (ALP) concentrations: Serum Ca concentration of chicks was not significantly ($p>0.05$) affected by both phytase and dietary Ca and nPP levels at 28 d (Table 4). Serum P level was significantly ($p<0.05$) increased in chicks fed 300 or 600 FTU phytase in compare to control birds. Furthermore, serum P concentration of the chicks was significantly ($p<0.05$) decreased by low Ca and nPP diet (Table 4). As shown in Table 5, serum P concentration was increased as the level of phytase was increased in both chicks fed normal and low Ca and nPP levels. The level of this blood parameter in chicks fed with normal Ca and nPP diet was higher than chicks fed low Ca and nPP diet. The level of ALP in chicks fed 600 FTU phytase was significantly ($p<0.05$) more than control and 300 FTU phytase (Table 4). There were not significant differences ($p>0.05$) between chicks fed 0 or 300 FTU phytase on this blood parameter. Low dietary Ca and nPP level significantly was increased ALP concentration. Highest level of this serum enzyme belonged to chicks fed with low dietary Ca and nPP level supplemented with no phytase. The level of ALP was decreased as the level of supplemented phytase was increased in both chicks fed normal and low Ca and nPP level diets (Table 5).

Carcass and yield cost: Eviscerated carcass weight (Carcass without head, neck, feet and gut) to live body weight of the chicks was not significantly different ($p>0.05$) among the treatments (Table 6 and 7). Neither phytase nor Ca and nPP level and their interaction had a significant effect ($p>0.05$) on organs relative weight to eviscerated carcass weight (e.g. liver, heart, gizzard plus proventriculus, thighs, wings and abdominal fat pad; data not shown. Minor pectoralis muscle had no significant difference ($p>0.05$) among the treatments too. However, major pectoralis muscle percentage in chicks fed low Ca and nPP diet supplemented with 600 FTU phytase was significantly ($p<0.05$) more than chicks fed normal Ca and nPP with the 3 phytase supplementation levels (Table 7). Yield cost calculated as Iranian Rial (RLS) feed

Table 4: Effects of microbial phytase and dietary Ca and nPP levels on blood factors and tibia ash percentage of 28-day-old broiler chickens

Treatment	Serum Ca (mg dL^{-1})	Serum P (mg dL^{-1})	Serum alkaline phosphatase (U/L)	Tibia ash (%)
Phytase (FTU/kg diet)				
0	6.67	5.86^{b}	4953.33^{a}	38.40^{b}
300	6.36	7.14^{a}	3794.00^{b}	39.62^{b}
600	6.64	7.86^{a}	3286.00^{c}	40.20^{a}
SE	0.164	0.345	47.9937	0.465
Ca and P levels				
Normal Ca and P	6.68	7.52^{a}	3827.10^{b}	39.94
Low Ca and nPP	6.62	6.26^{b}	4206.51^{a}	39.07
SE	0.133	0.279	38.828	0.377

$^{a-c}$Means within each column with no common superscript differ significantly ($p<0.05$). ^{SE}Standard Error

Table 5: Interaction between microbial phytase and dietary Ca and nPP levels on blood factors and tibia ash percentage of 28-day-old broiler chickens

Ca and P levels	Phytase (FTU/kg diet)	Serum Ca (mg dL^{-1})	Serum P (mg dL^{-1})	Serum alkaline phosphatease (U/L)	Tibia ash (%)
Normal Ca and P	0	6.69^{a}	7.21^{ab}	4216.50^{b}	39.25^{b}
	300	6.65^{ab}	7.32^{ab}	3902.66^{c}	39.86^{b}
	600	6.67^{ab}	7.98^{a}	3631.60^{d}	40.75^{ab}
Low Ca and P	0	6.64^{ab}	4.20^{c}	5684.00^{a}	37.26^{c}
	300	6.61^{ab}	6.90^{b}	3659.25^{d}	39.41^{b}
	600	6.62^{ab}	7.78^{a}	3017.00^{e}	40.50^{ab}
SE	-	0.239	0.504	69.99	0.664

$^{a-e}$Means within each column with no common superscript differ significantly ($p<0.05$). ^{SE}Standard Error

Table 6: Effects of microbial phytase and dietary Ca and nPP levels on apparent retention of Ca, tP and N (42 day), feed cost per kg of weight gain (42 day), Pectoral muscles relative weight to eviscerated carcass (%) and relative weight of carcass to live body weight (42 day)

	Apparent retention (%)				Pectoral muscle (%)		
Treatment	tP	Ca	N	Feed cost (RLS/KgWG)	Major	Minor	Carcass (%)
Phytase (FTU/kg diet)							
0	33.65^{c}	50.29^{c}	52.34^{b}	4630.4^{a}	11.99	3.51	70.464
300	40.40^{b}	57.98^{b}	58.53^{a}	4440.2^{b}	12.13	3.55	70.643
600	47.78^{a}	64.77^{a}	62.83^{a}	4440.1^{b}	12.33	3.57	71.564
SE	1.987	1.354	1.999	4.094	0.356	0.101	0.766
Ca and P levels							
Normal Ca and P	37.40^{b}	55.52^{b}	55.22^{b}	4550.2	12.46	3.38	70.108
Low Ca and nPP	44.49^{a}	59.84^{a}	59.58^{a}	4450.9	12.81	3.70	71.672
SE	1.622	0.950	1.632	3.343	0.291	0.082	0.625

$^{a-c}$Means within each column with no common superscript differ significantly ($p<0.05$). ^{SE}Standard Error

Table 7: Interaction between microbial phytase and dietary Ca and nPP levels on apparent retention of Ca, tP and N (42 day), feed cost per kg of weight gain (42 day) and relative weight of carcass to live body weight gain (42 day)

		Apparent retention (%)				Pectoral muscle (%)		
Ca and P levels	Phytase (FTU/kg diet)	tP	Ca	N	Feed cost (RLS/KgWG)	Major	Minor	Carcass (%)
Normal Ca and P	0	30.08^{e}	48.35^{e}	50.99^{c}	4610.6^{a}	11.31^{b}	3.46	69.25
	300	37.25^{bc}	56.05^{cd}	55.64^{bc}	4470.8^{b}	11.51^{b}	3.35	70.92
	600	44.85^{ab}	62.14^{ab}	59.02^{abc}	4460.2^{b}	11.56^{b}	3.45	70.14
Low Ca and P	0	39.22^{b}	52.22^{de}	53.69^{bc}	4550.1^{ab}	12.60^{ab}	3.68	72.03
	300	43.56^{ab}	59.92^{bc}	61.42^{ab}	4420.6^{b}	12.70^{ab}	3.66	70.00
	600	50.70^{a}	67.40^{a}	66.64^{a}	4400.0^{b}	13.11^{a}	3.76	72.98
SE		2.810	1.646	2.827	5.791	0.504	0.143	0.266

$^{a-e}$Means within each column with no common superscript differ significantly ($p<0.05$). ^{SE}Standard Error

cost per Kg of weight gain. Phytase significantly ($p<0.05$) decreased yield cost (RLS feed cost/Kg of weight gain) of both chicks fed with 300 and 600 FTU phytase diets. There was not significant difference between chicks fed with low or normal Ca and nPP diet (Table 6). Lowest yield cost was obtained by chicks fed low Ca and nPP supplemented with 600 FTU phytase and highest yield cost was obtained by chicks fed normal Ca and nPP with no phytase supplementation (Table 7).

DISCUSSION

The NRC (1994) sets the requirements of Ca and nPP, 10, 4.5 and 9 and 3.5 g kg^{-1} of diet for 0-3 and 3-6 wk-old broilers, respectively, to meet the optimal growth of the birds. In the present study, nPP and Ca levels were below the requirements to provide a more favorable situation for measuring the effect of phytase supplementation on increasing the bioavailabilities of the phytate P, Ca and N. The high content of phytate P in corn and soybean meal

leads to limited availabilities of P, Ca and N for poultry fed corn-soybean meal diets (Nelson *et al*., 1968; Dilger *et al*., 2004). Nelson *et al*. (1968) first reported that microbial phytase greatly improved utilization of phytate P when supplemented in broiler diets. Phytase has also been reported to improve the utilization of phytate and Ca (Simons *et al*., 1990). Phytic acid, a cation chelator, makes Ca unavailable for intestinal absorption. Phytase releases Ca from the insoluble salts of phytic acid and potentially makes Ca available for absorption in birds. Strong evidence indicates that microbial phytase is highly effective in degrading phytate (Simons *et al*., 1990; Denbow *et al*., 1995). Kornegay *et al*. (1996) reported that 735 FTU of phytase/kg of diet was equivalent to 1 g of nonphytate P for broilers fed corn-soybean meal diets and that about 20-60% of phytate P was hydrolyzed by graded levels of supplemental phytase. Findings of the present study are in agreement with the results reported by Kornegay *et al*. (1996). Graded levels of phytase increased Ca and tP retention by up to 28.8 and 42%, at 300 and 600 FTU, respectively (Table 6). That increased utilization of phytate P and Ca results in increased Ca and P retention is also supported by our findings of an increase in tibia ash. The differences between Ca and P retentions in the experiment of Kornegay *et al*. (1996) and the present study may be due to lower phytase levels used at the present study. Lower levels of Ca and nPP, regardless of phytase level, increased the retention of Ca and P up to 7.8 and 19%, respectively. Calcium is thought to be a key factor influencing the activity of mucosal phytase in small intestines of poultry and rat (Wise, 1983).

Phytase addition to diet increased ADG, ADFI and G:F ratio in this study and the increase in ADG was due to both increase in ADFI (numerical increase) and feed efficiency. These results are in agreement with observations of Dilger *et al*. (2004) and Payne *et al*. (2005) who reported that phytase increased ADFI of broiler chicks and are contrary to observations of Ravindran *et al*. (2001). Studies have reported that the response to phytase on ADG and ADFI was more pronounced in the normal Ca and nPP diets (Cabahug *et al*., 1999; Keshavarz, 2000; Watson *et al*., 2005) that is in agreement with findings of the present study. As seen in this study, deficiencies of Ca and nPP increased ADG, ADFI and G:F in chicks. This is in accord with observations of Yan *et al*. (2005) too.

Tibia ash percentage was also improved by phytase in both normal and low Ca and nPP diets and the effect of phytase on tibia ash percentage was not pronounced when Ca and nPP were reduced in the diet. Improvement in tibia ash observed in the present study is in accord with findings of Denbow *et al*. (1995), Sohail and Roland (1999) and Onyango *et al*. (2004). In chicks, it has been extensively reported that phytase addition to corn-soybean-based diets permits tP levels to be reduced without impairing bone ash (Yan *et al*., 2001; Dilger *et al*., 2004; Onyango *et al*., 2004, 2005; Payne *et al*., 2005) and only Rama-Rao *et al*. (1999) disagreed. Nevertheless, the level of tP reduction according to broiler age in phytase-supplemented diets lacks consensus.

The effects of Ca and nPP Level and phytase supplementation on apparent availability of Ca, tP and N are presented in Table 6 and 7. The main effects data indicated that the decrease in Ca and nPP content in the diet significantly increased ($p<0.05$) P, Ca and N retention by 18.96, 7.8 and 7.9%, respectively. Ca, P and N retention significantly were improved ($p<0.05$) by low dietary Ca and nPP level. Increase in P retention is in accord with findings of Viveros *et al*. (2002). But they reported that Ca retention was decreased by 8.4 % with low nPP diet while we observed that Ca retention was increased by low dietary Ca and nPP level. Our results were similar to those obtained by Ravindran *et al*. (2000) in chickens and Keshavarz (2000) in pullets, which indicated that the birds have a greater ability to retain P from diets with lower rather than higher nPP content. Compared to the normal Ca and nPP diet, the birds fed with low Ca and nPP diets without phytase had increased ($p<0.05$) P retention up to 30.4% but the differences for Ca and N was not significant ($p<0.05$). As seen in Table 7 and as expected, phytase effect on apparent retention of the minerals in low Ca and nPP level diet was more prominent than normal diet which agrees with the results of previous studies on chickens (Sebastian *et al*., 1996; Qian *et al*., 1997; Ravindran *et al*., 2000).

The effects of Ca and nPP concentrations and phytase supplementation on serum Ca and P levels and ALP activity are summarized in Table 4 and 5. These data show clearly that serum P, concentration responded in relation to dietary Ca and nPP levels. Phytase supplementation to a low Ca and nPP diet increased ($p<0.05$) serum P level by 16.7%. This increase in serum P was greatest in the low Ca and nPP diet and reached the level of serum P obtained in the normal Ca and P diet. This effect has also been reported in chickens (Sebastian *et al*., 1996; Rama-Rao *et al*., 1999). Neither phytase supplementation nor dietary Ca and nPP level had a significant effect on serum Ca concentration. Similarly, Sebastian *et al*. (1996) reported that serum Ca concentration was not affected by lowering the dietary Ca

level to 0.6% in a C- SBM diet. Similar findings have been reported by Viveros *et al.* (2002) and Onyango *et al.* (2004) who reported increased levels of serum P but decreased Ca in chicks when microbial phytase was supplemented to P deficient diets. Serum P in the present study seemed to reflect the P level of the diets. ALP concentration were reduced by phytase level ($p<0.05$). Total serum ALP measures a composite of several isoenzymes of Zn metalloenzymes by cells in a number of organs (liver, bone, muscle, small intestine and kidney) (Moss, 1982). We observed a 9.02% increase in serum ALP activity as dietary Ca and nPP decreased. These results agree with those reported by Viveros *et al.* (2002) in chickens. The increase of ALP activity may be induced by osteoblast activity, which is greater in young, growing animals and in disorders in which growth or remodeling of bone is taking place. The phytase supplementation decreased ($p<0.05$) serum ALP activity by 23.4, 33.7 for 300 and 600 FTU phytase/Kg of diet, respectively. This decrease was greater in low Ca and nPP diet, resulting in a Ca and nPP ×phytase ($p<0.05$) interaction effect. Similar results have been reported by Huff *et al.* (1998) in chickens. The decrease in serum ALP activity associated with the diets supplemented with phytase might reflect the down regulation of this enzyme resulting from the increased availability of phosphorus (Huff *et al.*, 1998). However, Roberson and Edwards (1994) showed that plasma ALP activity was not affected by phytase in broiler chicks.

Phytase levels in both the low and normal Ca and nPP levels, significantly improved BW, ADG, FCR, Ca, P and N retention, tibia ash percentage, serum P concentration and also feed cost per kg of weight gain ($p<0.05$). Some workers have found that microbial phytase has a positive influence on the utilization of nutrients other than P, such as amino acids (Ravindran *et al.*, 1999, 2000; Onyango *et al.*, 2005). Phytase supplementation to the low Ca and nPP diets increased ($p<0.05$) Ca, P and N retention at 3 and 6 week of age. As expected, phytase supplementation to the low Ca and nPP diets increased the P and Ca retention, which agrees with the results of previous studies on chickens (Sebastian *et al.*, 1996; Qian *et al.*, 1997; Ravindran *et al.*, 2000).

Phytase had no significant effect on relative weights of body organs and eviscerated carcass to live body weight ($p>0.05$). Ravindran *et al.* (2001) showed that dietary phytase increases amino acid availability. Lysine is one of most important amino acids to accretion of breast muscle. This response to phytase may be responsible for the increase in breast muscle yield seen in the present study.

CONCLUSION

In conclusion, as confirmed by physiological parameters, the enhancement of chick performance by dietary microbial phytase supplementation could be related to improved Ca, P and N retentions and to circulating serum concentration of P. Likewise, ALP activity was affected by Ca and nPP levels or phytase supplementation. Tibia ash was increased by phytase addition. The results obtained in this study suggest that phytase increases the availability and use of minerals for growth. It would be necessary to reevaluate Ca and P requirements of broiler chickens when the diet is supplemented with phytase. Generally, the best broiler performance was obtained by low Ca and nPP diet that supplemented with 600 FTU/kg of diet.

REFERENCES

AOAC, 2003. Official methods of analysis, Association of Official Analytical Chemists.17th Edn. Arlington, VA.

Cabahug, S., V. Ravindran, P.H. Selle and W.L. Bryden, 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. Br. Poult. Sci., 40: 660-666.

Denbow, D.M., V. Ravindran, E.T. Kornegay, Z. Yi and R.M. Hulet, 1995. Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poult. Sci., 74: 1831-1842.

Dilger, R.N., E.M. Onyango, J.S. Sands and O. Adeola, 2004. Evaluation of microbial phytase in broiler diets. Poult. Sci., 83: 962-970.

Gordon, R.W. and D.A. Roland, 1997. Performance of commercial laying hens fed various P levels with and without supplemental phytase. Poult. Sci., 76: 1172-1177.

Gordon, R.W. and D.A. Roland, 1998. Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. Poult. Sci., 77: 290-294.

Harland, B.F. and D. Oberleas, 1999. Phytic acid complex in feed ingredients. In Phytase in Animal Nutrition and Waste Management: A BASF Reference. BASF Corp., Mount Olive, NJ., pp: 69-75.

Huff, W.E., P.A. Moore, P.W. Waldroup, A.L. Waldroup, J.M. Balog, G.R. Huff, N.C. Rath, T.C. Daniel and V. Raboy, 1998. Effect of dietary phytase and available phosphorus corn on broiler chicken performance. Poult. Sci., 77: 1899-1904.

Jongbloed, A.W. and P.A. Kemme, 1990. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 1. Digestible phosphorus in feedstuffs from plant and animal origin. Neth. J. Agric. Sci., 38: 567-575.

Keshavarz, K., 2000. Reevaluation of nonphytate phosphorus requirement of growing pullets with and without phytase. Poult. Sci., 79: 1143-1153.

Kies, A.K., 1999. Phytase-Mode of action. In Phytase in Animal Nutrition and Waste Management: A BASF Reference. BASF Corp. Mount Olive, NJ., pp: 205-212.

Kornegay, E.T., D.M. Denbow, Z. Yi and V. Ravindran, 1996. Response of broilers to graded levels of microbial phytase added to maize-soybean-meal diets containing 3 levels of nonphytate phosphorus. Br. J. Nutr., 66: 251-259.

Mitchell, R.D. and H.M. Edwards, 1996. Effect of phytase and 1, 25-dihydroxycholecalciferol on phytate utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poult. Sci., 75: 95-110.

Moss, D.W., 1982. Alkaline phosphatase isoenzymes. Clin. Chem., 28: 2007-2016.

Nelson, T.S, J.J. McGillivray, T.R. Shieh, R.J. Wodzinski and J.H. Ware, 1968. Effect of phytate on the calcium requirement of chicks. Poult. Sci., 47: 1985-1989.

Nelson, T.S., 1967. The utilization of phytate phosphorus by poultry-A review. Poult. Sci., 46: 862-871.

National Research Council, 1994. Nutrient requirements of Poultry. 8th Rev. Edn. National Academy Press, Washington DC.

Onyango, E.M., M.R. Bedford and O. Adeola, 2004. The yeast production system in which Escherichia coli phytase is expressed may affect growth performance, bone ash and nutrient use in broiler chicks. Poult. Sci., 83: 421-427.

Onyango, E.M., M.R. Bedford and O. Adeola, 2005. Efficacy of an evolved scherichia coli phytase in diets of broiler chicks. Poult. Sci., 84: 248-255.

Payne, R.L., T.K. Lavergne and L.L. Southern, 2005. A comparison of two sources of phytase in liquid and dry forms in broilers. Poult. Sci., 84: 265-272.

Qian, H., E.T. Kornegay and D.M. Denbow, 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol and the calcium: Total phosphorus ratio in broiler diets. Poult. Sci., 76: 37-46.

Rama Rao, S.V., V. Ravindra Reddy and V. Ramasubba Reddy, 1999. Enhancement of phytate phosphorus availability in the diets of commercial broilers and layers. Anim. Feed Sci. Technol., 79: 211-222.

Ravindran, V., E.T. Kornegay, L.M. Potter, B.O. Ogunabameru, M.K. Welten, J.H. Wilson and M. Potchanakorn, 1995. An evaluation of various response criteria in assessing biological availability of phosphorus for broilers. Poult. Sci., 74: 1820-1830.

Ravindran, V., S. Cabahug, G. Ravindran, P.H. Selle and W.L. Bryden, 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci., 41: 193-200.

Ravindran, V., D.J. Cadogan, M. Cabahug, W. Bryden and P.H. Selle, 1999. Effects of phytic acid on the performance of poultry and swine. In Phytase in Animal Nutrition and Waste Management: A BASF Reference. BASF Corp. Mount Olive, NJ., pp: 93-99.

Ravindran, V., P.H. Selle, G. Ravindran, P.C.M. Morel, A.K. Kies and W.L. Bryden, 2001. Microbial phytase improves performance, apparent metabolizable energy and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci., 80: 338-344.

Roberson, K.D. and H.M. Edwards, 1994. Effects of ascorbic acid and 1, 25-dihydroxycholecalciferol on alkaline phosphatase and tibial dyschondroplasia in broiler chickens. Br. Poult. Sci., 35: 763-773.

SAS Institute, 1994. SAS User's Guide: Statistics, Version. 6.12th Edn. SAS Inst., Inc., Cary, NC.

Sebastian, S., S.P. Touchburn, E.R. Chavez and P.C. Lague, 1996. Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. Poult. Sci., 75: 1516-1523.

Sebastian, S., S.P. Touchburn, E.R. Chavez and P.C. Lague, 1997. Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. Poult. Sci., 76: 1760-1769.

Simons, P.C.M., H.A.J. Versteegh, A.W. Jongsbloed, P.A. Kemme, P. Slump, K.D. Bos, M.G.E. Wolters, R.F. Beudeker and G.J. Verschoor, 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr., 64: 525-540.

Sohail, S.S. and D.A. Roland, 1999. Influence of supplemental phytase on performance of broilers four to 6 weeks of age. Poult. Sci. 78: 550-555.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. McGraw-Hill Inc., New York.

Viveros, A., A. Brenes, I. Arija and C. Centeno, 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci., 81: 1172-1183.

Waldroup, P.W., J.H. Kersey, E.A. Saleh, C.A. Fritts, F. Yan, H.L. Stilborn, R.C. Crum and V. Raboy, 2000. Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poult. Sci., 79: 1451-1459.

Watson, B.C., J.O. Matthews, L.L. Southern and J.L. Shelton, 2005. The interactive effects of Eimeria acervulina infection and phytase in broiler chicks. Poult. Sci., 84: 910-913.

Wise, A., 1983. Dietary factors determining the biological activities of phytates. Nutr. Rev., 53: 791-806.

Yan, F., J.H. Kersey and P.W. Waldroup, 2001. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poult. Sci., 80: 455-459.

Yan, F., R. Angel, C. Ashwell, A. Mitchell and M. Christman, 2005. Evaluation of the broilers ability to adapt to an early moderate deficiency of phosphorus and calcium. Poult. Sci., 84: 1232-1241.