Middle-East Journal of Scientific Research 11 (11): 1481-1490, 2012
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2012.11.11.6468

The Effects of Phytase on Performance, Serum Mineral Levels, Enzyme Activities and Immune Function of Broilers Fed Nutritionally Marginal Diets

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Abstract: An experiment was conducted to study the effect of microbial phytase (Natuphos 10000) supplementation in chicks fed Nutritionally Marginal Diets on performance, plasma minerals, serum enzyme activities and humoral immunity. Treatments were replicated with 4 pens of 12 chicks each. Diets were Corn-wheat-soybean meal based with the same nutritional specifications, differing only in the concentration of Ca and nonphytate P (Ca-nPP). The treatments were: 1) adequate-Ca-nPP diets (CTL+); 2) Low-Ca-nPP diets (CTL-); 3 to 5 = diet 2 plus 600, 800, or 1000 phytase units (FTU) /kg of diet from Natuphos. The low-Ca-nPP diets caused a negative effect on feed consumption compared to the CTL+ diet. Performances of chicks fed with low-Ca-nPP diets and phytase were comparable to those obtained with the low-Ca-nPP and adequate-Ca-nPP diets. By decreasing Ca-nPP levels in the diet, plasma Ca concentrations, aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activity were reduced and alkaline phosphatase (ALP) activity increased. Phytase supplementation increased plasma Ca level and serum AST activity and reduced ALT, ALP and LDH activities. Antibodies against Newcastle disease virus vaccine were enhanced of 14 to 42-d-old broilers in the low Ca-nPP diets with phytase addition. Increasing the phytase dose to 1,000 FTU /kg did not improve immune function further than 800 FTU /kg. The results suggest that application of phytase in nutritionally marginal diets could enhance antibody titer of 14- to 42-d-old broilers, suggesting that both deficient in Ca-nPP and phytase may have a role in immune competence.

Key words: Phytase · Performance · Serum Mineral Levels · Enzyme Activities · Humoral Immunity · Broiler

INTRODUCTION

Feed ingredients of plant origin contain a number of components that cannot be digested by monogastric species. Examples of such antinutritive components include phytic acid (PA) in wheat. It contains 28.2% of bound P and represents, on average, 70% of the total P (TP) in the feed ingredients commonly used in poultry diets [1], a form poorly available to poultry. Phytic acid is present in grains and seeds as a mixed salt, phytate, which refers to the phytic acid molecule chelated to mineral cations, proteins, starch, lipids, or both starch and lipids [2]. Chickens are lacking or limited in phytase, the enzyme necessary for breakdown of the phytate molecule and subsequent release of phytate-bound phosphorus in plant feedstuffs. The dietary addition of feed phosphates not only increases the feed and production cost, but may also lead to an increase of soluble P in the litter resulting in the potential for water contamination from excess P in the soil. Phytates also reduce the availability of dietary carbohydrates and amino acids in pigs and poultry, either directly through interaction with digestive enzymes or by interaction with proteins. In addition, Cowieson *et al*. [3] reported that ingestion of phytic acid by poultry could increase the excretion of endogenous compounds, which may further impair the performance of poultry and exacerbate nutrient requirements. Therefore, phytate may be considered an antinutritional factor because it reduces the digestibility of phytate-chelated nutrients. To counteract the antinutritional effects of phytic acid,

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various alternatives have been proposed, including methods to improve phytate-bound P utilization by broilers and methods to reduce the phytate content of feed ingredients. Among these alternatives, one of the most practical and effective methods is the addition of microbial phytase. Adding appropriate exogenous enzymes to the feed can improve the extraction of nutrients from the feed, thereby decreasing feed costs, improving bird performance and decreasing the environmental impact of manure application to land [4].

Phytases are phosphatases capable of hydrolyzing one or more phosphate groups from the PA molecule yielding lower myo-inositol phosphates, inositol and inorganic P. Supplementation of diets with microbial phytase increases availability of phytate P and Zn, Ca, Mg, Cu and Fe in chicks. [5]. Numerous studies have demonstrated that dietary phosphorus levels in broiler diets can be reduced by supplementation with phytase. However, later studies demonstrated that the benefits of using dietary phytases are not restricted to improving mineral retention, but also may improve performance, energy and amino acid availability [3].

Formulation of broiler diets consists of an array of ingredients that match a desired nutrient profile at the minimum cost. The nutrient profile used is based on research or field observations evaluating the economically important production function of interest. This production function is typically body weight, feed conversion, or breast meat accretion, not immunity or disease resistance. Although the balance of nutrients is directly involved in optimizing the production function, variation in their levels can have a substantial impact on immune systems. Although some nutrient needs for broiler immunity are known, nutrient needs for immune responses in broiler chickens in environmental conditions that mimic field observations. The trace metals that have been associated with an improvement in immunity, or functions that support immunity, are: Zn, Mn, Cu, P and Se.

Pirgozliev *et al*. [6] has also demonstrated that supplementing diets with phytase significantly reduces the endogenous secretions, measured as sialic acid (SA), from the gastrointestinal tract (GIT) of broiler chickens. Sialic acid is a generic term for a family of acidic monosaccharide found at the terminal ends of sugar chains attached to cell surfaces and to soluble glycoproteins [7]. An increased concentration of SA is often associated with health problems such as cellular senescence, bacterial infections (e.g. Campylobacter), certain pathological conditions and osmotic fragility. It has been hypothesized that reduced GIT secretion– and thus improved gut health in the presence of phytase is one mechanism involved in the mode of action of dietary phytases [6]. However, the extraphosphoric effects of such dephosphorylation of phytate are not well elucidated and warrant further study because endogenous secretion, the GI tract microflora and the immune status of the host may be expected to be involved. It is of interest to understand the effect of phytase on the health and immune status of broilers. It is speculated that the degradation products of the action of phytase on phytate may regulate immunocyte activity [8] and this may be particularly true for broilers fed diets that contain a high concentration of phytate. Phytase can partially ameliorate the adverse effects of phytate in the GIT of broilers and may improve mucosal immunity by enhancing nutrient uptake for the intestinal immune cells and improving mucin integrity [9]. Moreover, previous experiments were usually conducted with corn- soybean meal - based diets and did not continue to market age (42 d). This was partially because endogenous phytase activity from wheat is higher than from corn, so the improvement of specific phytase addition to wheat-based diets could be higher. Therefore, the objective of this study was to evaluate, for a corn- wheat-soybean meal-based diet, the effect of low levels of Ca and nPP diets supplemented with microbial phytase (Natuphos) on broiler performance and antibodies against Newcastle disease virus (NDV) vaccine up to 42 d of age.

MATERIALS AND METHODS

A completely randomized experimental design was used and chicks (Ross 308) were divided into five treatment groups, with four replicates per treatment and 12 chicks (not sexed) per replicate. A total of 240, 1-d-old broiler chicks (Ross 308) were raised in floor pens with *ad libitum* access to feed and water and controlled ventilation. Chicks of a uniform body weight were placed in individual pens and Average initial body weight was 48 g. Temperature was maintained at 32°C for the first 4 d and then gradually reduced according to normal management practices until a temperature of 22°C was achieved at d 28. The light regimen was 23 h of light and 1 h of dark. All diets were formulated to provide 2900 kcal of ME/kg and to meet the amino acid ratios and all other nutrients as suggested by the NRC (1994) for broilers from 0 to 6 wk of age (Table 1), differing only in the concentration of dietary Ca and nPP. During the experiment, no antibiotics were offered to broilers via either feed or water. A positive control, adequate (Adq) in Ca and nPP without phytase

Table 1: Composition of experimental diets[1]

	Starter (0-21 d)					Finisher (22-42)				
Ingredients %	T1	T 2	T 3	T 4	T 5	T 1	T 2	T 3	T 4	T 5
wheat	30	30	30	30	30	30	30	30	30	30
Corn	24.64	25.97	25.964	25.964	25.964	35.07	35.91	35.904	35.902	35.90
Corn gluten	3.02	2.69	2.69	2.69	2.69	0	0	0	0	0
Wheat bran	5.21	7	7	7	7	5.1	4.97	4.97	4.97	4.97
Soybean	28.52	28.40	28.40	28.40	28.40	24.61	24.37	24.37	24.37	24.37
Soybean oil	3	3	3	3	3	2.16	2.10	2.10	2.10	2.10
Oyster shell	1.62	1.17	1.17	1.17	1.17	1.73	1.27	1.27	1.27	1.27
Ca phosphate	1.41	0.78	0.78	0.78	0.78	1.08	0.51	0.51	0.51	0.51
Salt	0.35	0.35	0.35	0.35	0.35	0.24	0.24	0.24	0.24	0.24
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lys	0	0	0	0	0	0.05	0.05	0.05	0.05	0.05
Met	0.13	0.14	0.14	0.14	0.14	0.08	0.08	0.08	0.08	0.08
Natuphose FTU/kg[4]	0	0	600	800	1000	0	0	600	800	1000
Nutrient composition Calculated										
ME, kcal/kg	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein %	21	21	21	21	21	18.1	18.1	18.1	18.1	18.1
Ca %	0.9	0.63	0.63	0.63	0.63	0.85	0.59	0.59	0.59	0.59
Available P %	0.4	0.28	0.28	0.28	0.28	0.32	0.2250	0.2250	0.2250	0.2250
P (Total) %	0.6911	0.4837	0.4837	0.4837	0.4837	0.41	0.41	0.41	0.41	0.41
Na %	0.18	0.18	0.18	0.18	0.18	0.1350	0.1350	0.1350	0.1350	0.1350
Lys%	0.99	0.99	0.99	0.99	0.99	0.91	0.91	0.91	0.91	0.91
Met %	0.45	0.45	0.45	0.45	0.45	0.3516	0.3516	0.3516	0.3516	0.3516
Met + Cys %	0.81	0.81	0.81	0.81	0.81	0.65	0.65	0.65	0.65	0.65
Trp %	0.2390	0.2425	0.2425	0.2425	0.2425	0.2122	0.2114	0.2114	0.2114	0.2114
Thr %	0.7182	0.7174	0.7174	0.7174	0.7174	0.6179	0.6175	0.6175	0.6175	0.6175
Analyesed										
P (total)	0.71	0.53	0.53	0.53	0.53	0.47	0.47	0.47	0.47	0.47
Ca	1.01	0.77	0.77	0.77	0.77	0.95	0.72	0.72	0.72	0.72

and negative control, Low in Ca and nPP without phytase were used. The treatments were: diet 1) adequate level of Ca and nPP (Adq Ca-nPP) as positive control (CTL+); diet 2) reduced levels of Ca and nPP (low Ca-nPP) as negative control (CTL-); diets 3 to 5) diet 2 plus 600, 800, or 1000 phytase units/kg of diet from NAT. The compositions of the diets are presented in Table 1. The microbial phytase (Natuphos 10000 Granulate) contained 10,000 FTU/ g phytase activity. The enzyme (Natuphos 10000; BASF Group, Ludwigshafen, Germany) was added to the diets in powder form and all diets were fed as mash.

Body weight gain (BWG) and feed consumed per pen basis were recorded weekly. Mortalities were recorded daily. At 42 d of age, three birds were randomly selected from each pen and blood samples were obtained from the wing veins for subsequent determination of minerals (Ca, P) in plasma and aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), in serum. To determine the contents of Ca, P samples of feed were dry-ashed [10]. Concentrations of minerals were measured at specific wavelengths for each element (Ca, 317.933; P, 214.914 nm) by using an inductively coupled plasma emission spectrometer. Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions of each element. Blood plasma and serum were analyzed for minerals (Ca, P), AST, ALT, ALP and LDH using an ADVIA 1650 chemistry system of Bayer (Bayer diagnostic, Puteaux, France).

At the age of 9 days, all chicks were vaccinated with Hitcher B1 NDV vaccine by eye dropper and bivalent killed vaccine (NDV plus AI) by inoculation according to the recommendation of the manufacturer (Newpasol 102, Inactivated W/O Emulsion ND + AI Vaccine, Pasouk

Biological Co). Blood samples were collected every week from the wing veins of individual chickens in all groups and their sera were separated and inactivated at 56°C for 30 min and kept at -20°C until analysis for the level of NDV antibody. Serum Antibody titer was measured by hemagglutination-inhibition test as described by Alexander *et al*. [11] on d7, 14, 21, 28, 35 and 42.

Statistical Analysis: When the chicks reached 42 d of age, the feeding trial was terminated. Data were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software (SAS Institute 2002). The treatment means with significant differences were compared by using Duncan's new multiple range tests. All statements of differences were based on significance at $P \leq 0.05$.

- [1]Calculated from NRC (1994).
- [2]provides per kilogram of diet: Cu (CuSO4_5 H2O), 4.0 mg; I (potassium iodate), 1.0 mg; Fe (ferrous sulfate_7 H2O), 60 mg; Mn (manganese sulfate_H2O), 60 mg; Se (sodium selenite), 0.1mg; Zn (zinc sulfate_7H2O), 44 mg; and Ca (calcium carbonate), 723 mg. For experiment 3, provides per kilogram of diet: Cu (CuSO4_5 H2O), 7.0 mg; I (potassium iodate), 1.0 mg;Fe (ferrous sulfate_7 H2O), 50 mg; Mn (manganese sulfate_H2O), 100 mg; Se (sodium selenite), 0.15 mg; and Zn (zinc sulfate_7H2O), 75 mg.
- [3]For experiments 1 and 2, provides per kilogram of diet: vitamin A (vitamin A palmitate), 4,500 IU; vitamin D3, 450 IU; vitamin E (vitamin E acetate), 50 IU; menadione (menadione sodium bisulfite), 2.4 mg; vitamin B12, 0.02 mg; biotin (D-biotin), 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; Ca-pantothenate, 20 mg; pyridoxine (pyridoxine_HCl), 6.4 mg; riboflavin, 15 mg; and thiamin (thiamin_HCl), 15.2 mg. For experiment 3, provides per kilogram of diet: vitamin A (vitamin A palmitate), 8,000 IU; vitamin D3, 3,000 IU; vitamin E (vitamin E acetate), 25 IU; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B12, 0.02 mg; biotin (D-biotin), 0.1 mg; folacin (folic acid), 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin (thiamin(HCl), 3 mg.
- [4]FTU = phytase units. One unit of phytase activity is the quanitity of enzyme required to produce 1 *i*mol of inorganic P per min from 5.1 micromoles/L of sodium phytate at a pH of 5.5 and a water bath temperature of 37°C.

RESULTS

The chickens were healthy throughout the experiment, with a mortality of less than 1 % that was unrelated to dietary treatment. No bacterial or viral disease infection was detected. The effects of Ca and nPP concentrations and phytase supplementation on growth performance are summarized in Table 2. In the growing phase and Overall, broilers fed the adequate Ca and P diet had increased feed intake ($P < 0.01$) compared with those fed the low Calcium and Phosphorus diets. The effect of Adq Ca-nPP diets on the same parameters as above in broilers fed the low Ca-nPP diet is shown in Table 2. Supplementation with phytase improved the feed intake (FI), BWG and feed conversion ratio(FCR) of birds ($P < 0.01$), but no differences were found between phytase at 800 and 1,000 FTU/kg of feed. In the starting, growing and overall phase, feed intake was increased ($P < 0.01$) in broilers fed NAT compared with those fed the low Ca-nPP diets. In this study, phytase also had a positive effect on FI in chicks fed a nutritionally adequate diet, but not significantly. These data indicate that phytase increases Performances of chicks fed with deficient in Ca and nPP in diets for broiler chicks.

The effects of Ca-nPP concentrations and phytase supplementation on plasma mineral levels and enzyme activities are summarized in Table 3. Ca content in plasma decreased as dietary Ca and P level decreased. However, P concentration in plasma was not affected by Ca and P level in the diet. Phytase supplementation to low Ca and P diets increased plasma Ca level by 16.3 %. There was no effect of phytase on plasma phosphorus. When all diets were considered, serum P was not significantly affected by either low-phosphorus and adequate Ca-nPP, but phytase supplementation increased serum P level from 4.27 mg/dL with no phytase to 5.76mg/dL with 1000 FTU /kg of phytase.

Decreasing dietary Ca-nPP levels caused a decrease in serum AST activity by 27.94% at 6 wk of age. This effect was counteracted by dietary phytase addition. Likewise, serum ALT activities were not affected by decreasing dietary Ca-nPP. However, LDH activities in birds fed a low- Ca-nPP diet were significantly reduced (up to 31.94%; $P < 0.01$) in comparison to the adequate Ca-nPP diet. Dietary phytase addition decreased serum ALT ($P < 0.01$) activities by 9.23 %. We observed a 6.58% increase in serum ALP activity as dietary Ca-nPP decreased. The phytase supplementation decreased ($P < 0.01$) serum ALP activity by 15.95%.

Table 2: Effects of Ca-nPP concentrations and phytase supplementation on the performance of broilers fed nutritionally marginal diets[1]

	Treatment[2]				
Item	1	2	3	4	5
			Starter (0 to 21d)[3]		
FI (g)	948 ± 11.58 [ab]	923.75 ± 21.5 [b]	959.83 ±8.65 [ab]	976.10 ± 24.2 [ab]	980 ±17.2 [c]
BWG (g)	545.75 ±8..33 [b]	513.75 ± 14.91[b]	607.98 ±11.40[a]	626.75 ±7.88[a]	635 ±14.21 [a]
FCR (g/g)	1.740 ±0.07 [a]	1.805 ± 0.051[a]	1.581 ± 0.11 [b]	1.543 ± 0.08 [b]	1.544 ± 0.09 [b]
			Grower (22 to 42 d)		
FI (g)	3556.50 ±54.2 [a]	3325.50 ±24.8[b]	3558.25 ± 65.1[a]	3680.75 ±48.5 [a]	3646.50 ±37.9 [a]
BWG (g)	1652.40 ±54.9 [bc]	1585.75 ±28.6 [c]	1741.75 ±68.2 [b]	1904.25 ±45.8 [a]	1985.75 ±33.8 [a]
FCR (g/g)	2.185 ±0.11 [a]	2.099 ±0.18 [a]	2.045 ±0.09 [ab]	1.933 ±0.15 [bc]	1.862 ±0.16 [c]
			Overall (0 to 42 d)		
FI (g)	4504.5 ± 81.6 [a]	4249.25 ± 121.6 [b]	4918 ±92.4 [a]	4647.85 ±75.8[a]	4626.50 ±125.6 [a]
BWG (g)	2198.15 ±54.3 [c]	2099.50 ±92.7 [c]	2349.73 ±55.7 [b]	2531 ±49.7 [a]	2395.75 ±77.6 [a]
FCR (g/g)	2.053 ±0.14 [a]	2.027 ±0.08 [a]	1.924 ±0.05 [b]	1.836 ±0.10 [bc]	1.748 ±0.07 [c]

a-c values within a row with no common superscript differ significantly (P < 0.05)

[1]Data are means of 4 replicates of 12chicks each.

[2]T 1) adequate-Ca-nPP diets (CTL+); T 2) Low-Ca-nPP diets (CTL-); T 3 to T 5 = diet 2 plus 600, 800, or 1000 phytase units (FTU) /kg of diet from Natuphos

[3]FI = feed intake BWG = body weight gain FCR = feed conversion ratio

Table 3: Effect of phytase level on serum biochemical parameters of broilers.

	Treatment				
Factor	T 1[1]	T 2	T 3	T 4	T 5
Ca (mg/100 mL)	7.65 ±0.65 [ab2]	6.75 ±0.95 [b]	7.85 ±1.2 [ab]	9.275 ± 0.88 [ab]	9.65 ± 1.6 [b]
P (mg/100 mL)	4.950 ± 0.41	4.275 ±0.25	5.475 ±0.54	5.678 ± 0.34	5.762 ±0.51
AST (U/L)	267.5 ± 11.54 [ab]	192.75 ± 8.95 [f]	222.5 ± 5.87[de]	249.75 ± 14.21 [c]	259.5 ± 8.67 [bc]
ALT (U/L)	2.46 ± 0.15 [b]	2.23 ±0.24[bc]	1.47 ± 0.12 [e]	1.76 ± 0.09[de]	1.88 ± 0.27 [d]
LDH (U/L)	1557.5 ±65.8 [b]	1060 ± 115.4[e]	1261.25 ± 84.7 [c]	1227.25 ± 101.8 [dc]	1106 ± 74.6 [de]
ALP (U/L)	3087.25 ±204.8[b]	3305 ±144.7 [a]	2670 ± 204.8[c]	2805 ± 55.4[c]	2983.75 ± 109.5 [b]

a-e Within comparisons, means with common superscript do not differ significantly (P < 0.01)

[1]T 1) adequate-Ca-nPP diets (CTL+); T 2) Low-Ca-nPP diets (CTL-); T 3 to T 5 = diet 2 plus 600, 800, or 1000 phytase units (FTU) /kg of diet from Natuphos.

[2]Data are means of 12 chicks for each treatment.

Table 4: Effect of phytase level on NDV vaccine antibody titer of broilers.

	Treatment				
Age (day)	T 1[1]	T 2	T 3	T 4	T 5
7	6.95 ± 0.35	6.95 ± 0.18	7.30 ± 0.38	7.27 ± 0.27	7.37 ± 0.41
14	5.12 ± 0.17[bdc]	4.7 ± 0.14 [d]	5.77 ± 0.13 [a]	5.52 ± 0.27 [abc]	5.72 ± 0.27 [ab]
21	4.45 ± 0.15 [a]	3.77 ± 0.11 [b]	4.42 ± 0.21 [a]	4.5 ± 0.21 [a]	4.87 ± 0.17 [a]
28	4.87 ±0.18 [c]	4.12 ±0.21 [c]	5.45 ±0.17 [ab]	6.02 ± 0.14 [a]	6.15 ±0.34 [a]
35	5.37 ± 0.21[bc]	5 ± 0.18 [c]	5.87 ±0.14 [ab]	6.25 ± 0.09 [a]	6.12 ±0.28 [a]
42	5.75 ± 0.31[abc]	4.87 ±0.24 [c]	5.12 ±0.24 [bc]	6.12 ±0.34 [a]	6.62 ±0.32 [a]

a-c values within a row with no common superscript differ significantly (P < 0.01)

[1]T 1) adequate-Ca-nPP diets (CTL+); T 2) Low-Ca-nPP diets (CTL-); T 3 to T 5 = diet 2 plus 600, 800, or 1000 phytase units (FTU) /kg of diet from Natuphos.

The effects of treatments on antibody production against NDV in broilers from day 7 to day 42 are presented in Table 4. On the 7th day of the study, there was no difference among antibody titers of experimental groups. Chickens of CTL- treatment showed reduction in antibody titers against NDV as compared to the CTL+. For both the low- and adequate Ca-nPP diets, antibody production against NDV was significantly increased by dietary phytase (P<0.01). However, for antibody titers, phytase was more effective in the deficient Ca and P diet than in

the CTL+ diet (Table 4). The increase in phytase dose rate from 800 to 1,000 FTU/kg of feed did not show any further improvements in anti-NDV antibodies. These results demonstrated the positive influence of phytase on the response to vaccination of the chickens' immune system

DISCUSSION

The effects of Low-Calcium and Phosphorus Diets and phytase supplementation on growth performance are summarized in table 2. Compared to the normal-Calcium and Phosphorus diet, the birds fed with low Calcium and Phosphorus diets without phytase had decreased weight gain (15.1 and 13.6 %;) and feed consumption (9.2; $P < 0.01$ and 10.1%; $P < 0.01$), at 3 and 6 wk age, respectively. In the entire growing period (0 to 6 wk), we also observed depressions in weight gain (14.0 %;) and feed consumption (4.1 %; $P < 0.01$). Although, there were no significant differences in Body weight gain ($P > 0.05$) during any phase of growth between the broilers fed the low-Calcium and Phosphorus diet compared with those fed the Adq Ca-nPP diet. This effect of P deficiency also has been reported by Punna and Roland [12]. Body weight was probably not a good indicator of the effects produced by reduced-P diets plus phytase with respect to a control diet. Indeed, Dhandu and Angel [13] reported that BWG was not a sensitive indicator of mineral sufficiency in broilers.

In the current study, the application of phytase to nutritionally marginal diets improved FI and BWG of broilers; results that are in agreement with previous work [14]. Phytase supplementation improved feed consumption at 3 and 6 wk of age ($P < 0.01$) and for the entire period ($P < 0.01$). Performance of chicks fed supplemental phytase with 0.28% (0 to 3 wk) and 0.23% (3 to 6 wk) nPP were higher than chicks fed the control diet that contained normal levels of nPP (0.4 and 0.32%, respectively). These values agree with the findings of Ahmad *et al*. [15]. It might be due to changes in the viscosity of the diets or transit time through the digestive tract of the chicken. The growth-promoting effect of P caused by phytase can be partially attributed to the increased concentrations of myo-inositol, the final product of phytate desphosphorylation and to the release minerals and trace elements from complexes with phytic acid. Similarly, it could also be due to a possible increase of starch digestibility or to an increased availability of protein [2].

Data also showed that feed consumption was affected at any stage by addition of phytase. In contrast, other authors have reported that addition of phytase did not affect feed consumption [15]. Phytase is able to liberate phytate-bound P and make more P available to the animals. Studies have reported improvements in performance when phytase was used in chickens [16]. In this study, phytase was used in Corn-wheat -soybean meal diets that were marginally deficient in both P and Ca. Because phytase acts on the phosphate groups associated with the inositol ring of phytic acid backbone and thus releases P and Ca, it is expected that the use of phytase would result in improved performance of the animal if P and Ca is the nutrient limiting for growth. In the current study, addition of phytase produced a significant improvement in performance above the CTL-, demonstrating that P and Ca was a limiting nutrient in the current study.

The Phytase efficacy in this study may be affected by Ca level in the diet. Researchers had reported a negative impact of higher Ca levels on phytase efficacy [17] but had not explored the full extent of this effect or how dietary Ca level differentially affects the efficacy of phytases. Sebastian *et al*. [17] reported that when a corn-soybean diet was supplemented with microbial phytase, the best phytase efficacy was seen with diets containing 0.6% Ca compared with those containing 1% Ca. cut In the entire growing period (0 to 6 wk), phytase supplementation in the low Ca-nPP diets, significantly improved weight gain and feed efficiency compared with the Adq Ca-nPP diets ($P < 0.01$; Table 2). Keshavarz [18] also reported a positive effect of phytase on BWG of pullets fed nutritionally adequate diets and suggested that the increase in BWG might have been due to an effect on digestibility of certain ingredients and provided the birds with adequate nutrients that otherwise could have been limiting for optimum growth. A more viscous diet prolongs feed passage time, which decreases feed intake. Watson *et al* [19] also reported that Phytase decreased transit time in chicks fed diets deficient in Ca and nPP on d 1. The results of our study suggest that the increase in BWG in chicks fed diets containing phytase was due to an increase in feed intake. This increase in feed intake might have been due to a faster transit time in chicks fed diets containing phytase. The chicks fed phytase ate more and thus gained more weight, regardless of the adequacy of the diet. As a result, the amounts of phytase enzyme and inorganic P needed in diets could be minimized resulting in reduced cost, lower P excretion levels and decreased environmental impact.

A plasma level of P is a result of the homeostatic regulation of P and significant lowering of these levels may be indicative of low body P reserves [20]. Compared to the Adq Ca-nPP diets, the birds fed with Low Calcium and Phosphorus Diets without phytase had decreased plasma Ca and P levels at 42 days of age but not significantly. Our results were similar to those obtained by Ravindran *et al*. [5] in chickens which indicated that the birds have a greater ability to retain P from diets with lower rather than higher nPP content. A possible explanation could be that the higher content of Ca relative to P in the low P diets caused an increase of intestinal pH and reduced the soluble fraction of minerals or that the decreased retention of minerals was related to bone mobilization to maintain serum P and excretion of excess Ca. It is possible that when P is limiting, more P is retained in the body for maintaining physiological functions, thus resulting in less P being excreted in the waste [21]. Phytase supplementation to the low- Ca -nPP diets increased Ca ($P< 0.05$) and P values at 6 wk of age. As expected, phytase supplementation to the low Ca and P diets increased plasma Ca and p values, this agrees with the results of previous studies on chickens [5]. Phytate-bound P liberated by phytase is available in the gut to be absorbed to maintain normal P homeostasis. This result may be due to the fact that phytase supplementation to the low nPP diet increases the Ca content, resulting in an efficient use of this mineral by birds. However, at higher nPP levels, Ca is bonded to phytate, therefore it cannot be fully retained by the bird, which leads to excessive excretion of Ca.

Decreasing dietary nPP levels caused a decrease in serum AST activity by 12.1% at 6 wk of age. This effect was counteracted by dietary phytase addition. In contrast to mammals, activity of AST is not liver-specific in birds [22]. Elevated activities usually indicate liver or muscle damage, but no particular significance is associated with low AST activity. Likewise, serum ALT and LDH activities were not affected by decreasing dietary Ca- nPP. However, LDH activities in birds fed a low Ca and nPP diet were significantly reduced ($P < 0.05$) in comparison to the normal Ca -nPP diet. Dietary phytase addition decreased serum ALT ($P< 0.01$) and increased LDH ($P <0.01$) activities by 27.9 and 6.16%, respectively. Plasma ALT activity has been reported to be low in all tissues of chickens [23], but ALT activities often increase due to damage in many tissues [24]. Therefore, specific diagnostic value of these enzymes in birds is poor. In many cases, birds with severe liver damage have normal ALT activities. Moreover, there are five LDH isoenzymes in birds; each occurs in a several tissues, including skeletal muscle, cardiac muscle, liver, kidney, bone and red blood cells [24]. The decrease in LDH activity that we observed may be related to liver diseases, because this enzyme decreases quickly as the disease progresses. 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). We observed a 9.1% increase in serum ALP activity as dietary nPP decreased. These results agree with those reported by Fernandes *et al*. [25] in chickens and could be related to intestinal lesions, skeletal disorders, or liver dysfunctions. 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. Cut The phytase supplementation decreased ($P<0.01$) serum ALP activity by 19.5%. 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 [26]. This decrease could also be related to the increase observed in Zn retention. Zn has a specific role in the reactivation of chicken intestinal ALP after acid exposure.

Antibodies are important biological agents prevalent in the healthy immune repertoire and they participate in the maintenance of immune homeostasis by exposure to environmental stimulation. It has been shown that low levels of humoral antibody may be related to disease susceptibility. In the current study, the addition of phytase to the diet increased ($p<0.01$) antibody production against NDV in broiler from 14 to 42 days of age. These results demonstrated the positive influence of phytase on the response to vaccination of the chickens' immune system. Ingestion of diets containing phytase resulted in higher titers, in comparison to birds which were fed diets without phytase, specifically during the weeks in which titers tended to decrease. No significant differences on antibody production against NDV were found between the phytase -treated birds in experimental period. In conclusion, phytate is a ubiquitous and potent antinutrient in monogastric diets and exerts a range of physiological, nutritional and immunological consequences on the host. Compensatory mechanisms are in place to allow normal digestive processes to continue, but these carry a substantial nutritional cost to the animal in terms of energy and amino acid and mineral requirements associated with synthesis, absorption, catabolism and autolysis. An understanding of the antinutritive effects of phytate is an important first step in

developing improved microbial phytases and in maximizing the potential of currently available phytase technology. Phytates may irritate the gut wall directly or by enhancing the growth of intestinal microflora, causing inflammation and provoking further immune response and increased production of cytokines [27]. However, activation of the immune system of the bird (e.g. increased production of antibodies in response of invading agents) is another energy-demanding process [28]. It has also been suggested that a strong immune response will increase the risk of tissue damage, increase the production of free radicals [29] and cause further deleterious effects on the organism. Studies in humans have demonstrated that lower molecular weight inositol phosphate esters are important in regulating vital cellular functions, such as ion channels and protein trafficking, oocyte maturation, cellular differentiation and may be involved in strengthening of the immune system by enhancing immunocyte activity and inhibiting pathological calcification [30]. Zyla *et al*. [31] reported that phytase addition to diets with a low P concentration enhanced the bursa weight of 21-d-old Hubbard broilers. Because the bursa is the source organ for B cells, the development of the bursa may induce the proliferation of B cells. Thus, the growth-promoting effect of phytase may be expressed via both nutrient release and a physiological regulation mechanism.

The investigation of innate mucosal humoral immunity by Liu, *et al*. [32] showed that the levels of SIgA were increased by phytase addition. The mucosal epithelium is a potential effectors tissue of integrated host responses, producing SIgA to protect GI-associated port of entry into the body. The degradation products of phytate by phytase may regulate immune activity of these cells [8]. Kettunen and Rautonen [33] reported that the use of xylanase, amylase and protease or a combination of the enzymes and betaine enhanced nutrient uptake by intestinal cells and concluded that the concentration of IgA in the digesta contributed to improvements in immune competence. Therefore, the fact that phytase addition ameliorates the excess secretion of mucin [3] may contribute to maintaining a "normal" gut ecology that enhances host immunity by stimulating the immunological defense mechanisms at the mucosal and systemic level, perhaps by reducing the concentration of saprogenic compounds.

In current study, anti-NDV antibodies were improved by phytase addition, indicating that dietary factors may affect specific immune responses. This effect of P deficiency also has been reported by Liu *et al*. [32]. There is some precedent for these effects in the literature; because Gao *et al*. [34] reported that supplementation of diets with nonstarch polysaccharide-degrading enzyme preparations significantly increased the anti-NDV titers of chicks. It also should be mentioned that the untreated phytate in the CTL- diets is likely to reduce the release of dietary minerals, starch and amino acids within the GIT and leads to an unbalanced nutrient supply in the intestinal lumen. The results suggest that application of phytase in nutritionally marginal diets could enhance growth performance and antibody production in broilers, suggesting that both phytate and phytase may have a role in performance and immune competence.

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