Effects of Microbial Phytase Supplementation on Mineral Utilization and Serum Enzyme Activities in Broiler Chicks Fed Different Levels of Phosphorus

A. Viveros,* A. Brenes,†[1] I. Arija,* and C. Centeno†

*Departamento de Producción Animal, Facultad de Veterinaria, Ciudad Universitaria, 28040 Madrid, Spain; and †Instituto de Nutrición y Bromatología, Facultad de Farmacia, Ciudad Universitaria, 28040 Madrid, Spain

ABSTRACT An experiment was conducted to study the effect of microbial phytase (Natuphos 500) supplementation in chicks (0 to 6 wk of age) fed different levels of nonphytate phosphorus (nPP) on performance, mineral retention, bone and plasma minerals and serum enzyme activities. Data were analyzed as a 2 × 2 factorial arrangement with two levels of nPP for age periods of 1-d-old to 3 wk (0.35 and 0.22%) and 3 to 6 wk (0.27 and 0.14%) and two levels of phytase (0 and 500 U/kg) in each period. A positive control, adequate in nPP and Ca without phytase, was used. The low-nPP diets caused a negative effect on the performance ($P < 0.05$) compared to the normal-nPP diet. Phytase had a favorable effect on weight gain at 3 wk ($P < 0.004$) and 6 wk ($P < 0.0475$) of age and on feed consumption only at 3 wk ($P < 0.0106$). Feed efficiency was not affected at any stage by addition of phytase. Performances of chicks fed with 0.35 and 0.27% nPP and phytase were comparable to those obtained with the normal-nPP diets. Decreasing nPP content in the diet increased ($P < 0.0001$) P retention at 3 and 6 wk of age, increased Mg retention at 6 wk, and decreased ($P < 0.0001$) Ca and Zn retentions at 3 and 6 wk, respectively. Phytase supplementation increased ($P < 0.0001$) Ca, P, Mg, and Zn retention at 3 and 6 wk of age. Likewise, the decrease in nPP content in the diet caused a significant reduction of tibia ash ($P < 0.0023$) and Mg content ($P < 0.0001$) in tibia ash and reduced liver ($P < 0.0240$), spleen ($P < 0.0176$), and tibia ($P < 0.0001$) weights. Similarly, Ca ($P < 0.0369$) and Zn ($P < 0.0181$) contents in tibia ash were increased in response to decreasing nPP levels in the diet. Phytase supplementation increased tibia weight ($P < 0.0019$), tibia ash ($P < 0.0021$), and Mg ($P < 0.0339$) and Zn ($P < 0.0353$) concentrations and reduced ($P < 0.0161$) the relative liver weight. By decreasing nPP levels in the diet, plasma Ca ($P < 0.0001$), Mg ($P < 0.0001$) and Zn ($P < 0.0048$) concentrations, and alkaline phosphatase (ALP) activity ($P < 0.0299$) increased, and plasma P content ($P < 0.0001$), aspartate aminotransferase (AST) activity ($P < 0.0001$), and total protein (TP) content ($P < 0.0050$) were reduced. Phytase supplementation increased plasma P level ($P < 0.0001$) and serum AST activity ($P < 0.0049$), reduced plasma Ca ($P < 0.0001$) and Mg ($P < 0.0050$) contents, and reduced serum alanine aminotransferase (ALT) ($P < 0.0048$), ALP ($P < 0.0001$) and lactate dehydrogenase (LDH) ($P < 0.0192$) activities. Plasma Zn was not affected by phytase supplementation. These results demonstrated that microbial phytase supplementation to low-P diets improved performance; P, Ca, Mg, and Zn use; and tibia weight and relative liver weight in broiler chickens. Likewise, serum AST, ALT, ALP, and LDH activities, as well as TP concentration, were also affected by phytase supplementation.

(*Key words*: nonphytate phosphorus, phytase, chick, mineral retention, bone mineral)

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INTRODUCTION

Phosphorus is the second most abundant mineral in the animal body, approximately 80% of which is found in the bones and teeth. As with calcium, the formation and maintenance of bone are quantitatively the most important functions. The 20% of P not present in the skeletal tissues is widely distributed in the fluids and soft tissues of the body, where it serves a range of essential functions (Underwood and Suttle, 1999). Approximately two-thirds of the total P in plants, which are the major constituents of poultry diets, is in the form of phytate (Punna and Roland, 1999; Viveros et al., 2000) and is unavailable or poorly utilized by humans and other monogastric ani-

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[1]To whom correspondence should be addressed: abrenes@inb.csic.es.

Abbreviation Key: AIA = acid-insoluble ash; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; nPP = nonphytate phosphorus; TP = total protein.

mals. This unavailability is due to the very low phytase activity found in the digestive tract (Pallauf et al., 1994). Therefore, diets of monogastric animals are often supplemented with sources of inorganic P to meet the P requirements of the animal, which increases the cost of the diets and contributes to environmental pollution.

Dietary supplementation with sources of microbial phytase is well established as an effective and practical method of improving phytate digestibility in production animals. Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. Phytases are naturally found in a number of seeds including cereals, legume, by-products, and other feedstuffs (Eeckhout and De Paepe, 1994; Viveros et al., 2000) and in microbial sources (Wyss et al., 1999). Supplementation of diets with microbial phytase increases availability of phytate P and Zn in chicks (Sebastian et al., 1996; Qian et al., 1997; Mohanna and Nys, 1999; Ravindran et al., 2000). Phytase also increases availability and retention of Ca (Sebastian et al., 1996; Qian et al., 1997) and improves absorption and retention of Mg, Cu, and Fe (Pallauf et al., 1992; Sebastian et al., 1996). In contrast, Roberson and Edwards (1994) showed that microbial phytase supplementation does not influence the Zn retention.

The status of bones, especially leg bones (tibia, femur, metatarsus), may have a direct impact on the quality of the poultry meat produced (Orban et al., 1999). Phosphorus deficiency resulting in breakage or defects of these bones during processing results in the downgrading of poultry meat. Therefore, manipulations of the P level in the diet, such as adding microbial phytase to improve P availability, have to be validated for their effects on bone status. Although live performance and mineral retention are important measures of any dietary changes, plasma and bone mineral concentrations are generally more sensitive than performance factors for evaluating minerals bioavailability. Due to the lack of information and contradictions among several authors, more studies that are comprehensive are needed to elucidate the efficacy of phytase in minerals use. Therefore, the aim of this experiment was to evaluate the influence of dietary phytase on the utilization of Ca, P, Mg, and Zn in broiler chickens receiving low-P diets. Growth performance, mineral retention, plasma mineral concentration, serum enzyme activities, and bone mineral served as the response criteria.

MATERIALS AND METHODS

Birds, Feeding, and Management

A total of 240, 1-d-old male broiler chicks (Cobb strain) were obtained from a commercial hatchery. The birds were housed in electrically heated stainless steel starter battery brooders in an environmentally controlled room with 23-h constant overhead fluorescent lighting for 3 wk. The chicks were allocated to 30 pens, each pen containing eight chicks, to receive five dietary treatments of corn-soybean-based diets with six replicates of each treatment. At the end of 3 wk, birds were moved from starter to grower-finisher stainless steel batteries for the remaining 3 wk.

The experiment consisted of a 2 × 2 factorial arrangement of the treatments with two nonphytate phosphorus (nPP) concentrations and two levels of supplemental phytase,[2] excluding the control group. The birds of the control group were fed a sequence of 0.45% nPP from 1 d of age to 3 wk (starter diets) and 0.37% nPP from 3 to 6 wk of age (grower-finisher diets). The nPP level was reduced by 0.1% during each period in Treatment 2 and by 0.13% during each period in Treatment 3 (Table 1). The birds of Treatments 4 and 5 were fed similar diets to Treatments 2 and 3, respectively, including 500 U phytase (Natuphos 500)/kg of diet. These dietary levels were formulated below the current NRC (1994) recommendations to ensure maximum responses with phytase. Diets in pellet form and water were provided ad libitum. Mortalities were recorded daily. At the end of each experimental period (3 or 6 wk), birds were weighed and feed consumption was recorded for feed efficiency computation. Experimental procedures were approved by the University Complutense of Madrid Animal Care and Ethics Committee in compliance with the Ministry of Agriculture, Fishery and Food for the Care and Use of Animals for Scientific Purposes.

Collection of Samples and Measurements

For determination of mineral retention (Ca, P, Mg, and Zn) at 19 and 40 d of age, clean stainless steel collection trays were placed under each cage (six per treatment), and feces from the birds were collected for 72 h. A subsample of feces was collected in polyethylene bags, weighed, and dried. Feces were mixed thoroughly, frozen at –20 C, and freeze-dried. Prior to chemical analysis, these samples were ground (0.5-mm, screen). Celite,[3] a source of acid-insoluble ash (AIA), was added at 10 g/kg to all diets as an indigestible marker. At 42 d of age, two birds were randomly selected from each pen, and blood samples were obtained by cardiac puncture for subsequent determination of minerals (Ca, P, Mg, and Zn) in plasma, and aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and total protein (TP) in serum. After the chicks were killed by cervical dislocation (42 d), the liver, spleen, and right tibiae were removed, cleaned from adhering tissue, and weighed using 12 randomly selected chicks (two per replicate) per treatment. For bone ash determination, tibiae were dried 110 C for 12 h, extracted with ether, dried again, and weighed. The dry fat-free bones were ashed in a muffle furnace at 550 C. Ash weight was calculated as a percentage of dry fat-free bone weight.

[2]Natuphos, BASF Corp., Mt. Olive, NJ.
[3]Celite Corp., Lompoc, CA.

TABLE 1. Ingredients and nutrient composition of experimental diets[1]

Ingredient	Starter (0 to 3 wk)					Grower-finisher (3 to 6 wk)				
	T_1	T_2	T_3	T_4	T_5	T_1	T_2	T_3	T_4	T_5
	(%)									
Corn	52.21	52.35	52.68	52.35	52.68	61.00	61.17	61.36	61.17	61.36
Soybean meal (48% protein)	37.43	37.52	37.64	37.52	37.64	29.11	29.18	29.27	29.18	29.27
Sunflower oil	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.53	4.51	4.53
Calcium carbonate	1.12	1.47	1.94	1.47	1.94	1.05	1.41	1.86	1.41	1.86
Dicalcium phosphate	1.94	1.36	0.58	1.36	0.58	1.59	0.99	0.24	0.99	0.24
NaCl	0.29	0.29	0.15	0.29	0.15	0.29	0.29	0.29	0.29	0.29
DL-Methionine (99%)	0.15	0.15	0.15	0.15	0.15	0.10	0.10	0.10	0.10	0.10
Trace minerals mix[2]	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamins mix[3]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Celite[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Natuphos 500[5] (U/kg diet)	–	–	–	500	500	–	–	–	500	500
Nutrient composition										
Calculated										
ME, kcal/kg	3,010	3,010	3,010	3,010	3,010	3,025	3,025	3,025	3,025	3,025
Crude protein (N × 6.25)	22.30	22.30	22.30	22.30	22.30	19.00	19.00	19.00	19.00	19.00
Lysine	1.25	1.25	1.25	1.25	1.25	1.04	1.04	1.04	1.04	1.04
Methionine + cystine	0.88	0.88	0,88	0.88	0,88	0.76	0.76	0.76	0.76	0.76
Calcium (Ca)	0.99	0.99	0.99	0.99	0.99	0.87	0.87	0.87	0.87	0.87
Total phosphorus (tP)	0.71	0.61	0.49	0.61	0.49	0.62	0.52	0.40	0.52	0.40
Nonphytate phosphorus	0.45	0.35	0.22	0.35	0.22	0.37	0.27	0.14	0.27	0.14
Ca:tP ratio	1.39:1	1.62:1	2.02:1	1.62:1	2.02:1	1.40:1	1.67:1	2.2:1	1.67:1	2.2:1
Analyzed										
Crude protein (N × 6.25)	22.10	22.08	22.19	22.08	22.19	18.66	18.88	18.67	18.88	18.67
Calcium	1.29	1.19	1.19	1.19	1.19	1.14	1.04	1.12	1.04	1.12
Phosphorus, total	0.81	0.71	0.58	0.71	0.58	0.76	0.64	0.52	0.64	0.52
Magnesium	0.20	0.19	0.20	0.19	0.20	0.18	0.18	0.18	0.18	0.18
Zinc (mg/kg)	100	92	91	92	91	112	101	92	101	92

[1]Birds in the control group (T_1) were fed 0.45% nonphytate P (nPP) from 1 d of age to 3 wk (starter diets) and 0.37% nPP from 3 to 6 wk (grower-finisher diets) without enzyme added. The nPP level was reduced by 0.1% in each period in T_2 and by 0.13% in each period in T_3. The birds of T_4 and T_5 were fed similar diets to T_2 and T_3, respectively, plus 500 U phytase/kg diet.

[2]Mineral mix supplied the following per kilogram of diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg.

[3]Vitamins mix supplied the following per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 1,000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B_{12}, 11.5 μg; riboflavin, 5.5 mg; Ca pantothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1,020 mg; folic acid, 0.75 mg; biotin, 0,25 mg; delaquin, 125 mg; DL-methionine, 500 mg.

[4]Celite Corp., Lompoc, CA.

[5]Natuphos 500 (BASF Corp., Mt. Olive, NJ) was used as the source of microbial phytase to provide 500 U phytase/kg of diet.

Chemical Analyses

Feed samples were ground in a hammer mill, passed through a 0.5-mm sieve, and analyzed for N content using the Kjeldahl procedure (AOAC, 1990). To determine the contents of Ca, P, Mg, and Zn, samples of feed, feces, and bones were dry-ashed (AOAC, 1990). Concentrations of minerals were measured at specific wavelengths for each element (Ca, 317.933; P, 214.914; Mg, 279.079; and Zn, 213,856 nm) by using an inductively coupled plasma emission spectrometer.[4] Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions[5] of each element. The AIA contents of diet and feces were measured after ashing the samples and treating the ash with boiling 4 *M* HCL (Siriwan et al., 1993). Blood plasma and serum were analyzed for minerals (Ca, P, Mg, and Zn), AST, ALT, ALP, LDH, and TP using a ADVIA 1650 chemistry system of Bayer.[6]

[4]Model JY-24, Jobin Yvon, Longjumeau, Cedex, France.
[5]Junsei Chemical Co., Ltd., Tokyo, Japan.
[6]Bayer diagnostic, Puteaux, France.

Calculations and Statistical Analyses

Ca, P, Mg, and Zn retentions were calculated using the following formula: 100% − [100% × (AIA concentration in feed/AIA concentration in feces) × (Ca, P, Mg, or Zn concentration in feces/Ca, P, Mg, or Zn concentration in feed)]. Data were analyzed as a 2×2 factorial arrangement with two levels of nPP for 1 d of age to 3 wk (0.35 and 0.22%) and for 3 to 6 wk (0.27 and 0.14%), and two levels of phytase (0 and 500 U/kg) in each period. Data were subjected to analysis of variance using the general linear models procedures of SAS software (SAS Institute, 1990). Treatment 1 was considered a positive control, adequate in nPP and Ca; therefore, phytase was not added. This control treatment was excluded from statistical analysis. The statistical model used was

$$Y_{ijk} = \mu + P_i + E_j + PE_{ij} + R_k + e_{ijk}$$

where Y_{ijk} is the individual observation, μ is the experimental mean, P_i is the nPP effect, E_j is the enzyme phytase effect, R_k is the replication effect, and e_{ijk} is the error term. Significant differences among treatment means were determined at $P < 0.05$ by Duncan's multiple-range test.

RESULTS AND DISCUSSION

Growth Performance

The effects of nPP concentrations and phytase supplementation on growth performance are summarized in Table 2. The main effects data indicated that the decrease in nPP content in the diet depressed weight gain (15.1 and 13.6%; $P < 0.0001$), feed consumption (9.2; $P < 0.0001$ and 10.1%; $P < 0.0012$), and feed efficiency (7; $P < 0.0001$ and 3.9%; $P < 0.0171$), 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%; $P < 0.0001$), feed consumption (9.9%; $P < 0.0003$), and feed efficiency (4.1%; $P < 0.0056$). Compared to the normal-nPP diet, the birds fed with low-nPP diets without phytase had decreased ($P < 0.05$) weight gain (up to 18.7%) and feed consumption (up to 14%) and increased feed efficiency (up to 5.9%) at 6 wk of age. This depression in growth was produced mainly between 0 and 3 wk of age and with the lowest nPP (0.22 and 0.14%) levels. This effect of P deficiency has also been reported by Qian et al. (1996a) and Punna and Roland (1999).

The inclusion of 500 U phytase/kg diet improved weight gain at 3 and 6 wk of age by 6.7% ($P < 0.0049$) and 6.1% ($P < 0.0475$), respectively, and for the entire period (6.3%; $P < 0.0097$). Phytase supplementation only increased ($P < 0.0106$) feed consumption (5.3%) at 3 wk of age. Performance of chicks fed supplemental phytase with 0.35% (0 to 3 wk) and 0.27% (3 to 6 wk) nPP were comparable with those of chicks fed the control diet that contained normal levels of nPP (0.45 and 0.37%, respectively). These values agree with the findings of Broz et al. (1994), Sebastian et al. (1996), Rama-Rao et al. (1999), and Ahmad et al. (2000). 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, as suggested by Knuckles and Betschsrt (1987), or to an increased availability of protein (Selle et al., 2000).

Data also showed that feed efficiency was not affected at any stage by addition of phytase, probably as a result of simultaneous increases in weight gain and feed consumption. These results were similar to those obtained by Perney et al. (1993), Sebastian et al. (1996), and Ahmad et al. (2000). In contrast, other authors have reported improved feed efficiency with supplemental phytase (Rama-Rao et al., 1999). The interaction between nPP content and phytase was not significant neither for different stages of growth nor for the entire period studied. However, Qian et al. (1996a) indicated that microbial phytase seems to be more efficient in diets with no or low levels of inorganic P supplementation in swine and poultry.

Mineral Retention

The effects of nPP concentrations and phytase supplementation on apparent availability of minerals are presented in Table 3. The main effects data indicated that the decrease in nPP content in the diet significantly increased ($P < 0.0001$) P retention by 12.6% and reduced ($P < 0.0001$) Ca retention by 8.4% at 3 wk. Likewise, P and Mg retention were significantly ($P < 0.0001$) increased by 10.3 and 9.7%, respectively, and Zn retention ($P < 0.0001$) was reduced by 48.2% at 6 wk in response to decreasing nPP level in the diet. Our results were similar to those obtained by Um and Paik (1999) in laying hens, 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. In contrast, Um and Paik (1999) reported an increased Zn retention in low-P diets.

Compared to the normal-nPP diet, the birds fed with low-nPP diets without phytase had decreased ($P < 0.05$) Ca, Mg, and Zn retentions at 3 (up to 30.0, 26.9, and 88.6%, respectively) and 6 wk of age (up to 25.3, 24.5, and 91.2%, respectively). P retention was increased (20.1%; $P < 0.05$) in the lowest nPP group at 3 wk of age and was reduced (7.3%; $P < 0.05$) at 6 wk in the intermediate-nPP level in comparison to the control diet. We have no obvious explanation why, in the absence of phytase, decreasing nPP content caused significant decreases of relative retentions of Ca, Mg, and Zn. 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 (Shafey, 1993) or that the decreased retention of minerals was related to bone mobilization to maintain serum P and excretion of excess Ca, Mg, and Zn.

Phytase supplementation to the low-nPP diets increased ($P < 0.0001$) Ca, P, Mg, and Zn retention at 3 (22,1, 4.9, 20.4 and 169%, respectively) and 6 wk of age (15.0, 10.1, 23,0, and 93.9%, respectively). As expected, phytase supplementation to the low-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).

However, although phytase supplementation increased Ca retention, this increase could not reach the level obtained in the normal-P diet. Perney et al. (1993) and Ahmad et al. (2000) reported that P excretion on the low-P diet decreased with the addition of phytase, which might have increased the availability of P and Ca. In fact, the results of the current study showed that fecal P and Ca concentrations were decreased by 6.1 and 7.1%, respectively, at 3 wk and by 6.3 and 2.7%, respectively, at 6 wk in chicks fed low-P diets plus phytase. 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 (Li et al., 2000).

Likewise, phytase supplementation increased the Zn and Mg retention, in spite of containing adequate Zn and Mg levels in the diet. The increase in Zn retention might have been due to greater availability of Zn from the phytate-mineral complex. Improvements in the utilization of Zn by supplemental phytase have also been reported in pigs (Pallauf et al., 1992; Adeola et al., 1995), chickens

TABLE 2. Effect of dietary nonphytate phosphorus (nPP) level with and without microbial phytase on weight gain, feed consumption, and feed efficiency of broiler chicks from 0 to 6 wk of age[1]

TRT[2]	nPP (%)		Phytase[3]	Weight gain (g)			Feed consumption (g)			Feed efficiency (g:g)			Mortality %
	0 to 3 wk	3 to 6 wk	(U/kg)	0 to 3 wk	3 to 6 wk	0 to 6 wk	0 to 3 wk	3 to 6 wk	0 to 6 wk	0 to 3 wk	3 to 6 wk	0 to 6 wk	0 to 6 wk
1[4]	0.45	0.37	–	631^{a}	1880^{a}	2511^{a}	890^{a}	3389^{a}	4279^{a}	1.41^{b}	1.80^{b}	1.70^{b}	2.33
2	0.35	0.27	–	592^{b}	1846^{a}	2438^{a}	843^{b}	3320^{a}	4163^{ab}	1.42^{b}	1.80^{b}	1.71^{b}	2.33
3	0.22	0.14	–	505^{c}	1537^{c}	2042^{c}	774^{c}	2904^{b}	3678^{c}	1.53^{a}	1.89^{a}	1.80^{a}	5.50
4	0.35	0.27	500	635^{a}	1894^{a}	2529^{a}	897^{a}	3393^{a}	4290^{a}	1.41^{b}	1.79^{b}	1.70^{b}	3.33
5	0.22	0.14	500	536^{c}	1695^{b}	2231^{b}	806^{bc}	3131^{ab}	3937^{b}	1.50^{a}	1.85^{ab}	1.76^{ab}	2.33
Pooled SEM				26.38	115.02	116.09	34.70	208.33	216.08	0.05	0.07	0.06	4.70
Main effects[5]													
nPP													
0.35 – 0.27				614^{a}	1870^{a}	2484^{a}	870^{a}	3357^{a}	4227^{a}	1.42^{b}	1.80^{b}	1.71^{b}	2.83
0.22 – 0.14				521^{b}	1616^{b}	2137^{b}	790^{b}	3018^{b}	3808^{b}	1.52^{a}	1.87^{a}	1.78^{a}	3.92
Phytase													
0				549^{b}	1692^{b}	2240^{b}	809^{b}	3112	3921	1.48	1.85	1.76	3.92
500				586^{a}	1795^{a}	2380^{a}	852^{a}	3262	4114	1.46	1.82	1.73	2.83
Source of variation				Probabilities									
nPP effect				0.0001	0.0001	0.0001	0.0001	0.0012	0.0003	0.0001	0.0171	0.0056	NS
Phytase effect				0.0049	0.0475	0.0097	0.0106	NS	NS	NS	NS	NS	NS
nPP × phytase				NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

$^{a-c}$Means in columns with no common superscript differ significantly ($P < 0.05$).

[1]Data are means of six replications of eight chicks.

[2]TRT = treatment.

[3]Natuphos (BASF Corp., Mt. Olive, NJ) was used to supply 500 U microbial phytase per kilogram of diet.

[4]Control group.

[5]Data were analyzed as a 2 × 2 factorial arrangement, excluding the control group.

TABLE 3. Effects of dietary nonphytate phosphorus (nPP) level with and without microbial phytase on Ca, P, Mg, and Zn retention in broiler chicks at 3 and 6 wk of age[1]

				Mineral retention (%)							
	nPP (%)			3 wk				6 wk			
TRT[2]	0 to 3 wk	3 to 6 wk	Phytase[3] (U/kg)	Ca	P	Mg	Zn	Ca	P	Mg	Zn
1[4]	0.45	0.37	–	52.83[a]	51.47[c]	30.60[ab]	38.53[b]	52.66[a]	55.34[b]	32.22[a]	31.50[a]
2	0.35	0.27	–	39.61[d]	51.47[c]	22.38[d]	22.19[d]	42.50[d]	51.30[c]	24.32[d]	18.33[c]
3	0.22	0.14	–	36.97[e]	61.81[a]	27.90[c]	4.39[e]	39.32[e]	56.41[b]	27.25[c]	2.77[d]
4	0.35	0.27	500	49.16[b]	57.68[b]	31.62[a]	27.15[c]	44.96[c]	56.32[b]	30.54[b]	22.53[b]
5	0.22	0.14	500	44.34[c]	61.16[a]	28.91[bc]	44.41[a]	49.15[b]	62.30[a]	32.90[a]	18.39[c]
Pooled SEM				1.73	1.23	1.72	1.13	0.96	0.89	0.92	1.08
Main effects[5]											
nPP											
0.35 - 0.27				44.38[a]	54.58[b]	27.00	24.67	43.73	53.81[b]	27.43[b]	20.43[a]
0.22 – 0.14				40.65[b]	61.48[a]	28.40	24.40	44.23	59.36[a]	30.08[a]	10.58[b]
Phytase											
0				38.29[b]	56.64[b]	25.14[b]	13.29[b]	40.91[b]	53.86[b]	25.79[b]	10.55[b]
500				46.75[a]	59.42[a]	30.26[a]	35.78[a]	47.05[a]	59.31[a]	31.72[a]	20.46[a]
Source of variation				Probabilities							
nPP effect				0.0001	0.0001	NS	NS	NS	0.0001	0.0001	0.0001
Phytase effect				0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
nPP × phytase				NS	0.0001	0.0001	0.0001	0.0001	NS	NS	0.0001

[a-e]Means in columns with no common superscript differ significantly ($P < 0.05$).

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

[2]TRT = treatment.

[3]Natuphos (BASF Corp., Mt. Olive, NJ) was used to supply 500 U microbial phytase per kilogram of diet.

[4]Control group.

[5]Data analyzed as a 2 × 2 factorial arrangement, excluding the control group.

(Sebastian et al., 1996), and laying hens (Um and Paik, 1999). However, Roberson and Edwards (1994) found that phytase did not affect zinc retention in broiler chicks. Moreover, the increase obtained in Mg retention by phytase supplementation may be caused by a decrease in endogenous Mg loss or by a significant reduction of Mg excretion. These values agree with the findings of Pallauf et al. (1992) in pigs and those of Um and Paik (1999) in laying hens. However, Brink et al. (1991) in rats and Adeola et al. (1995) and Pallauf et al. (1994) in pigs reported that addition of phytase did not affect Mg retention.

A significant interaction ($P < 0.0001$) was observed between nPP levels and phytase in P, Mg, and Zn retentions at 3 wk and in the case of Ca and Zn retentions at 6 wk. This interaction indicated that P and Mg retentions were greater (12.1 and 41.3%, respectively) when phytase was added to diets containing 0.35 and 0.27% nPP in comparison to 0.22 and 0.14% nPP level (–0.1 and 3.6%). However, phytase supplementation caused greater ($P < 0.0001$) Ca (25%) and Zn (564%) retention in diets containing lowest nPP level. In the case of P, this effect could be related to an excessive Ca:tP ratio in the lowest-nPP diets in comparison to the intermediate-nPP diets. A decreased phytate-P digestibility in response to increasing the level of dietary Ca or Ca:tP ratio has been reported in rats (Nelson and Kirby, 1979), poultry (Qian et al., 1996a), and pigs (Lei et al., 1994). In fact, Qian et al. (1997) showed that the increase observed in P retention by phytase addition was negatively influenced by increasing the dietary Ca:tP ratio, this effect being stronger at lower levels of available P. Presumably, increasing the concentration of a multivalent cation such as Ca would increase the formation of insoluble mineral-bound phytin crystals, which may be resistant to hydrolysis by phytase activities. Moreover, and probably more importantly, extra Ca could directly repress phytase activity by competing for the active sites of the enzymes (Pointillart et al., 1985).

Likewise, phytase supplementation caused a greater Ca retention at 6 wk of age in the birds fed with the lowest-nPP levels compared with the highest nPP levels. This result may be due to the fact that phytase supplementation to the lowest-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. Qian et al. (1996b) also demonstrated in turkeys that Ca retention increased when level of nPP decreased. As a last resort, demonstrating a response of Ca absorption to dietary phytase supplementation, independent of its effect on P, would be difficult, as the release of minerals bound to phytate by phytase is initiated by hydrolysis of phosphate (Lei and Stahl, 2000).

Bone Minerals and Organ Weights

The effects of nPP level and phytase supplementation on mineral concentrations in tibia ash and relative organ weights are summarized in Table 4. The main effect data indicated that the decrease in nPP content of a broiler diet caused, at 6 wk, a significant decrease ($P < 0.0023$) in tibia ash by 4.9%. These data agree with studies of Punna and Roland (1999), Fernandes et al. (1999), and Leeson et al. (2000) in chickens. However, Keshavarz (2000) did not observe differences in tibia ash being influ-

TABLE 4. Effects of dietary nonphytate phosphorus level (nPP) with and without microbial phytase on mineral bone and organ weights in broiler chicks at 6 wk of age[1]

	nPP (%)			Bone mineral					Organ weight					
TRT[2]	0 to 3 wk	3 to 6 wk	Phytase[3] (U/kg)	Tibia ash (%)	Ca (%)	P (%)	Mg (%)	Zn (μg/g)	Tibia (g)	Liver (g)	Spleen (g)	Tibia (g/100 g BW)	Liver (g/100 g BW)	Spleen (g/100 g BW)
1[4]	0.45	0.37	–	48.58^{a}	38.98	19.17^{a}	0.86^{a}	342^{ab}	6.76^{a}	62.60^{ab}	3.84^{a}	0.25	2.29^{b}	0.14
2	0.35	0.27	–	44.25^{b}	38.40	18.67^{ab}	0.82^{b}	317^{b}	5.98^{b}	62.05^{ab}	3.71^{ab}	0.24	2.45^{ab}	0.15
3	0.22	0.14	–	43.09^{b}	39.26	18.78^{ab}	0.76^{c}	360^{a}	5.18^{c}	59.14^{ab}	3.03^{b}	0.24	2.63^{a}	0.14
4	0.35	0.27	500	47.58^{a}	38.35	18.87^{ab}	0.85^{a}	356^{a}	6.65^{a}	65.63^{a}	3.68^{ab}	0.24	2.35^{b}	0.13
5	0.22	0.14	500	44.22^{b}	39.05	18.62^{b}	0.78^{c}	376^{a}	5.59^{bc}	57.72^{b}	3.44^{ab}	0.23	2.38^{b}	0.14
Pooled SEM				2.30	0.86	0.41	0.03	28.24	0.57	8.23	0.80	0.03	0.24	0.03
Main effects[5]														
nPP														
0.35 – 0.27				45.92^{a}	38.38^{b}	18.77	0.84^{a}	337^{b}	6.32^{a}	63.84^{a}	3.70^{a}	0.24	2.42	0.14
0.22 – 0.14				43.66^{b}	39.16^{a}	18.70	0.77^{b}	368^{a}	5.39^{b}	58.43^{b}	3.24^{b}	0.24	2.51	0.14
Phytase														
0				43.67^{b}	38.83	18.73	0.79^{b}	339^{b}	5.58^{b}	60.60	3.37	0.24	2.54^{a}	0.15
500				45.90^{a}	38.70	18.75	0.82^{a}	366^{a}	6.12^{a}	61.68	3.56	0.24	2.38^{b}	0.14
Source of variation				Probabilities										
nPP effect				0.0023	0.0369	NS	0.0001	0.0181	0.0001	0.0240	0.0176	NS	NS	NS
Phytase effect				0.0021	NS	NS	0.0339	0.0353	0.0019	NS	NS	NS	0.0161	NS
nPP × phytase				NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a-c]Means in columns with no common superscript differ significantly ($P < 0.05$).

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

[2]TRT = treatment.

[3]Natuphos (BASF Corp., Mt. Olive, NJ) was used to supply 500 U microbial phytase per kilogram of diet.

[4]Control group.

[5]Data analyzed as a 2 × 2 factorial arrangement, excluding the control group.

enced by nPP levels in the diets of pullets. Mineral content in tibia ash significantly increased Ca by 2% ($P < 0.0369$) and Zn by 9.2% ($P < 0.0181$) and decreased Mg by 9.1% ($P < 0.0001$) as dietary nPP level decreased. However, P concentration in tibia ash was not affected by nPP level in the diet.

Phytase supplementation to low-nPP diets significantly increased ($P < 0.0021$) tibia ash by 5.1%. This increase has been reported by several authors working with chickens and is considered a good indication of bone mineralization (Broz et al., 1994; Qian et al., 1996c; Sebastian et al., 1996; Ahmad et al., 2000; Leeson et al., 2000). The improvement in ash percentage in tibia can be related to the increase in Ca, P, Mg, and Zn retentions from the phytate-mineral complex by the action of phytase. In contrast, Keshavarz (2000) observed in pullets and Rama-Rao et al. (1999) in chickens that tibia ash was not influenced by phytase in the diets.

Likewise, phytase supplementation to low-nPP diets significantly increased the Mg ($P < 0.0339$) and Zn ($P < 0.0353$) concentrations by 3.8 and 8%, respectively. With Mg- and Zn-deficient diets, increase of these minerals in tibial ash by adding phytase has been demonstrated in turkeys (Qian et al., 1996b) and chickens (Qian et al., 1996c; Mohanna and Nys, 1999). However, in our experimental diets, Mg and Zn concentrations were higher than NRC (1994) recommendations for normal growth (600 and 40 mg/kg, respectively). It is possible that the requirement for obtaining a maximum Mg and Zn deposition in tibia ash is greater than the requirement needed for obtaining an adequate growth. In contrast, Qian et al. (1996c), Sebastian et al. (1996), and Ahmad et al. (2000) showed that phytase did not affect concentrations of any of the minerals measured in whole tibia ash. Finally, phytase supplementation did not cause any change in the concentration of Ca and P in tibia ash, in spite of improving Ca and P retention. Similar results have been reported by Broz et al. (1994). Hongxing et al. (1999) also found an increase in Ca and P digestibility that was not accompanied by an increase of these minerals in tibia ash. However, Leeson et al. (2000) observed an increase of P in tibia ash in chicks fed a phytase-supplemented diet.

The main effect data indicated that the decrease in nPP content of a broiler diet caused decreases in weights of tibia ($P < 0.0001$), liver ($P < 0.0240$), and spleen ($P < 0.0176$) at 6 wk, by 14.7, 8.5, and 12.4%, respectively. However, relative organ weights were not affected in response to decreasing nPP level in the diet. Compared to the normal-P diet, the birds fed with low-P diets without phytase showed a decrease ($P < 0.05$) in tibia weight (up to 23.4%) and an increase in relative liver weight (up to 14.8%). Leeson et al. (2000) also found in chickens an increase of tibia weight in response to decreasing nPP level in the diet. However, Keshavarz (2000) reported in laying hens that tibia weight was not influenced by dietary nPP level.

Phytase supplementation to low-nPP diets increased ($P < 0.0019$) the tibia weight by 9.7% and reduced ($P < 0.0161$) the relative liver weight by 6.3%. Spleen was not affected by phytase supplementation. The interaction between nPP levels and phytase was not significant for any of the tibia minerals or relative organ weights measured. The response of tibial weight to microbial phytase in the present study is similar to previous observations reported in chickens (Perney et al., 1993; Leeson et al., 2000) and ducks (Orban et al., 1999).

Plasma Minerals and Serum Enzyme Activities

The effects of nPP concentrations and phytase supplementation on plasma mineral levels and enzyme activities are summarized in Table 5. Decreasing nPP levels in the diet increased plasma Ca ($P < 0.0001$), Mg ($P < 0.0001$), and Zn ($P < 0.0048$) concentrations, at 6 wk, by 11.6, 17.4, and 8.8%, respectively, and a 27.5% decrease ($P < 0.0010$) in plasma P level. For Ca, similar results have been found by Sebastian et al. (1996) and Fernandes et al. (1999). As suggested by Taylor and Dacke (1984), the low-P diets caused an elevated ionized Ca in the plasma, which depressed the release of parathyroid hormone, thus reducing inhibition of parathyroid hormone on tubular reabsorption of phosphate and permitting urinary excretion of additional Ca absorbed from the gut during feeding of a low-P diet. Decreased plasma P has also been observed in pullets (Keshavarz, 2000), chickens (Fernandes et al., 1999), and turkeys (Atia et al., 2000) as dietary nPP level decreased.

Phytase supplementation to a low-nPP diets increased ($P < 0.0001$) plasma P level by 8%. As indicated by the significant nPP × phytase interaction, this decrease in plasma P was greatest in the low-nPP diet and did not reach the level of plasma P obtained in the normal-P diet. This effect has also been reported in chickens (Broz et al., 1994; Sebastian et al., 1996; Rama-Rao et al., 1999), ducks (Orban et al., 1999), and turkeys (Atia et al., 2000). Lei et al. (1994) showed that the increase observed in plasma P was negatively influenced by increasing the dietary Ca:tP ratio in pigs. Similarly, we obtained greater plasma P concentration in diets with Ca:tP at 1.67:1 in comparison to diets with Ca:tP at 2.2:1. Likewise, phytase supplementation reduced ($P < 0.0001$) plasma Ca level by 2.3%, in spite of obtaining an increase in Ca retention. Broz et al. (1994) and Sebastian et al. (1996) also found decreases in plasma Ca concentrations with phytase addition. Nevertheless, there are other studies that do not show a significant effect of phytase on plasma Ca concentration (Roberson and Edwards, 1994; Rama-Rao et al., 1999).

As with Ca, plasma Mg concentration was reduced (5.3%; $P < 0.0050$) by phytase supplementation, even though Mg retention was increased. Brink et al. (1991) also observed in rats a lack of correlation between Mg retention and plasma Mg concentrations. Plasma Zn content was not affected by enzyme addition. This lack of response has also been reported by Roberson and Edwards (1994) and Sebastian et al. (1996) in broiler chicks. These authors suggest that adequate Zn in a diet might be responsible for a lack of effect on plasma Zn. Most authors have reported increased plasma Zn when feeding

TABLE 5. Effect of dietary nonphytate phosphorus (nPP) level with and without microbial phytase on plasma total Ca, P, Mg, Zn, and total protein, and serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate deshidrogenase (LDH) activities in broiler chicks at 6 weeks of age[1]

	nPP (%)			Plasma minerals				Serum enzyme activities				
TRT[2]	0 to 3 wk	3 to 6 wk	Phytase[3] (U/kg)	Ca (mg/100 mL)	P (mg/100 mL)	Mg (mEq/L)	Zn (μg/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)	Total protein (g/100 mL)
1[4]	0.45	0.37	–	10.49^{d}	7.84^{a}	2.14^{c}	119^{c}	303^{a}	2.08^{ab}	2410^{c}	1269^{a}	2.91^{b}
2	0.35	0.27	–	10.85^{c}	7.33^{b}	2.13^{c}	138^{b}	235^{bc}	1.83^{ab}	2940^{b}	815^{bc}	3.04^{ab}
3	0.22	0.14	–	12.12^{a}	4.89^{d}	2.74^{a}	145^{ab}	200^{d}	2.33^{a}	3502^{a}	967^{b}	2.89^{b}
4	0.35	0.27	500	10.61^{d}	7.40^{b}	2.23^{c}	135^{b}	242^{b}	1.50^{b}	2620^{bc}	846^{b}	3.07^{a}
5	0.22	0.14	500	11.82^{b}	5.79^{c}	2.38^{b}	153^{a}	220^{cd}	1.50^{b}	2565^{bc}	639^{c}	2.91^{b}
Pooled SEM				0.16	0.28	0.15	13.42	24.74	0.75	444	221	0.17
Main effects[5]												
nPP												
0.35 – 0.27				10.73^{b}	7.37^{a}	2.18^{b}	137^{b}	239^{a}	1.67	2780^{b}	831	3.06^{a}
0.22 – 0.14				11.97^{a}	5.34^{b}	2.56^{a}	149^{a}	210^{b}	1.92	3034^{a}	803	2.90^{b}
Phytase												
0				11.49^{a}	6.11^{b}	2.44^{a}	142	218^{b}	2.08^{a}	3221^{a}	891^{a}	2.97
500				11.22^{b}	6.60^{a}	2.31^{b}	144	231^{a}	1.50^{b}	2593^{b}	743^{b}	2.99
Source of variation				Probabilities								
nPP effect				0.0001	0.0001	0.0001	0.0048	0.0001	NS	0.0299	NS	0.0050
Phytase effect				0.0001	0.0001	0.0050	NS	0.0049	0.0048	0.0001	0.0192	NS
nPP × phytase				NS	0.0001	0.0001	NS	NS	NS	0.0090	0.0052	NS

[a-d]Means in columns with no common superscript differ significantly ($P < 0.05$).

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

[2]TRT = treatment.

[3]Natuphos (BASF Corp., Mt. Olive, NJ) was used to supply 500 U microbial phytase per kilogram of diet.

[4]Control group.

[5]Data analyzed as a 2 × 2 factorial arrangement, excluding the control group.

Zn-deficient diets supplemented with phytase (Pallauf et al., 1994; Mohanna and Nys, 1999). According to Pallauf et al. (1994), the failure to observe increases in circulating Zn concentrations in conjunction with increases in Zn retention indicate that the maximum plasma Zn concentrations may not be a good indicator of absolute Zn availability.

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 (Lewandowski and Harrison, 1986). 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 nPP. However, LDH activities in birds fed a low-nPP diet were significantly reduced (up to 38.5%; $P < 0.05$) in comparison to the normal-nPP diet. Dietary phytase addition decreased serum ALT ($P < 0.0048$) and LDH ($P < 0.0192$) activities by 27.9 and 16.6%, respectively. Plasma ALT activity has been reported to be low in all tissues of chickens (Bogin and Israeli, 1976), but ALT activities often increase due to damage in many tissues (Zantop, 1997). 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 occur in a several tissues, including skeletal muscle, cardiac muscle, liver, kidney, bone, and red blood cells (Zantop, 1997). The decrease in LDH activity that we observed may be related to liver diseases, because this enzyme decreases quickly as the disease progresses. Electrophoretic separation of the isoenzymes can help establish the source of LDH activity.

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 9.1% increase in serum ALP activity as dietary nPP decreased. These results agree with those reported by Fernandes et al. (1999) in chickens and Atia et al. (2000) in turkeys 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. Campbell and Coles (1986) reported that the intestinal isoenzyme makes the largest contribution to serum ALP activity in birds. In contrast, Zantop (1997) indicated that increases of serum ALP are most related to liver diseases, even though the level of ALP activity in this organ is low.

The phytase supplementation decreased ($P < 0.0001$) serum ALP activity by 19.5%. This decrease was greater (26.8%) in lowest-nPP diet, resulting in a NPP × phytase ($P < 0.0090$) interaction effect. Similar results have been reported by Huff et al. (1998) in chickens and Atia et al. (2000) in turkeys. The decrease in serum ALP activity associated with the diets supplemented with phytase might reflect the downregulation of this enzyme resulting from the increased availability of phosphorus (Huff et al., 1998). 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. However, Watkins and Southern (1993) did not find differences in ALP activity by increasing Zn use. In addition, Roberson and Edwards (1994) showed that plasma ALP activity was not affected by phytase in broiler chicks. Quantification of the five isoenzymes of ALP in low-P diets may explain the source and the mode of action of these enzymes.

Serum TP concentration was reduced ($P < 0.0050$) by 5.2% as dietary nPP decreased. Dietary phytase supplementation had no effect on TP in serum. The decrease in serum TP could be due to a decrease of synthesis of protein caused by liver disorders, small intestinal malabsorption, or increased loss caused by proteinuria due to renal diseases or malnutrition (Zantop, 1997). In our experiment, the potential existence of protein-phytate complex in the gut of chick could affect the digestibility and absorption of protein (Selle et al., 2000) and modify the concentrations of TP in serum. Moreover, the liver could be affected, because P plays a vital role in metabolic functions (Underwood and Suttle, 1999).

In conclusion, as confirmed by several physiological parameters, the enhancement of chick performance by dietary microbial phytase supplementation could be related to improved Ca, P, Mg, and Zn retentions and to circulating plasma concentrations of Ca and P. Likewise, AST, ALT, ALP, and LDH activities, as well as TP, concentration were affected by nPP level or phytase supplementation. Tibia weight, tibia ash, and Mg and Zn contents in tibia ash were increased and relative liver weight was reduced by phytase addition. The results obtained in this study suggest that phytase modifies some serum enzyme activities and increases the availability and use of minerals for growth. It would be necessary to reevaluate mineral requirements of broiler chickens when the diet is supplemented with phytase.

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