The Effects of Supplemental Microbial Phytase on the Performance and Utilization of Dietary Calcium, Phosphorus, Copper, and Zinc in Broiler Chickens Fed Corn-Soybean Diets

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ABSTRACT A 3-wk feeding trial with 180 sexed day-old broiler chickens was conducted to study the efficacy of microbial phytase (Natuphos 1000) on growth performance, relative retention of P, Ca, Cu, and Zn, and mineral contents of plasma and bone. Treatments involved a normal P level corn-soybean diet, a low-P diet, and a low-P plus phytase (600 phytase units/kg) diet. Phytase supplementation increased ($P \leq 0.05$) body weight in male and female chickens by 13.2 and 5.8%, respectively, at 21 d. The improvements yielded body weights comparable to those obtained on the normal P diet. Phytase supplementation overcame ($P \leq 0.05$) the depression of feed intake observed on the low-P diet. Treatments had no effect on feed:gain ratio. Phytase supplementation of the low-P diet increased ($P \leq 0.05$) the relative retention of total P, Ca, Cu, and Zn by 12.5, 12.2, 19.3, and 62.3 percentage units, respectively, in male chickens. Microbial phytase increased the plasma P by 15.7% and reduced ($P \leq 0.05$) the Ca concentration by 34.1%, but had no effect on plasma concentrations of Cu or Zn. Phytase supplementation increased the percentage ash in both head and shaft portions of dry, fat-free tibia bone to a level comparable to that of the normal-P diet. Phytase supplementation had no effect on the concentration of any of the minerals measured in whole tibia ash but did increase ($P \leq 0.05$) the DM percentage of P and Ca in tibia head of male chickens by 0.65 and 1.4 percentage units, respectively. These results show that microbial phytase supplementation of a low-P diet increased growth and relative retention of total P, Ca, Cu, and Zn and improved bone mineralization in broiler chickens.

(*Key words*: phytase, broiler, phosphorus, calcium, zinc, copper)

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INTRODUCTION

Plant materials are the major constituents of poultry diets. About two-thirds of the P of plant origin is present as phytic acid in the form of myo-inositol phosphates (Cromwell, 1980). Phosphorus in the phytic acid form is poorly available to monogastric animals because they lack phytase, the enzyme that hydrolyzes phytic acid into inositol and orthophosphate (Peeler, 1972). It is well documented that microbial phytase supplementation improves the availability of phytate-bound P in broiler chickens (Nelson *et al.*, 1971; Simons *et al.*, 1990; Roberson and Edwards, 1994). However, there is little information about the availability of trace minerals when the broiler diet is supplemented with microbial phytase.

Phytate, being a strong acid can form various salts with the important minerals such as Ca, Mg, Cu, Zn, Fe, and K, thus reducing their solubility (Eardman, 1979). Nutritionally more important is the fact that maximum binding of Zn-Ca-Cu-phytate as well as Cu-Ca-phytate occurs at pH 6, which is the normal pH of the duodenum, where maximum absorption of divalent cations takes place (Oberleas, 1973). When phytic acid is hydrolyzed by microbial phytase it may release all phytate-bound minerals. However, in a very recent study, Aoyagi and Baker (1995) have shown that microbial phytase supplementation reduced the Cu utilization by 50% in chickens fed soybean meal. The authors speculated that phytase may have increased the Zn bioavailability in soybean meal and the released Zn might have had antagonized the absorption of Cu. In another study with broiler chickens, Roberson and Edwards (1994) have shown that phytase addition to a corn-soybean meal diet did not affect the Zn retention whereas phytase plus 1,25-dihydroxycholecalciferol [$1,25\text{-(OH)}_2D_3$] increased Zn retention. The lack of information and contradictions concerning the efficacy of phytase on the availability of trace minerals indicate the need for more investigation. Therefore, the objectives of this study were to determine the effects of microbial phytase supplementation on the performance of broiler chickens fed low-P corn-soybean meal diets,

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and to study the efficacy of microbial phytase on the apparent availability of Ca, P, and trace minerals such as Cu and Zn and on the mineral contents of bone and plasma.

MATERIALS AND METHODS

Experimental Design

A total of 180 sexed day-old Ross × Indian River broiler chicks were purchased from a commercial hatchery,[2] wing-banded, and weighed individually prior to the experimentation. Fifteen birds were assigned to each of 12 pens (6 pens of each sex) and housed in thermostatically controlled Petersime[3] battery brooders with raised wire floors. All birds had *ad libitum* access to water and experimental diet from day-old to 21 d and they received 24 h light/d. Each of three dietary treatments was replicated two times per sex. The experimental design was a completely randomized one with factorial arrangements of treatments. The experimental diets (Table 1) were as follows: 1) corn-soybean meal diet, no enzyme (control); 2) corn-soybean (low phosphate), no enzyme; 3) corn-soybean (low phosphate) plus phytase (a commercially available microbial phytase preparation, Natuphos 1000,[4] was added at 600 phytase units/kg diet). According to the manufacturer's instructions, 1 unit of phytase is defined as the quantity of enzyme which sets free 1 μmol of inorganic P/min from 0.0015 mol/L sodium phytate at pH 5.5 at 37 C. Individual body weights of chickens and group feed consumption data were recorded on Days 7, 14, and 21.

Apparent Availability of Minerals

On the first 3 d of Weeks 2 and 3 of the experiment, the daily feed consumption and total fecal output were recorded. A representative sample of excreta and feed from each pen was freeze-dried, ground, and analyzed for mineral content. Calcium, Cu, and Zn were determined by flame atomic absorption spectrophotometer[5] after wet ashing with HNO_3. Phosphorus was determined by the alkalimeter ammonium molybdate method (AOAC, 1984) and the color intensity read in a UV/VIS spectrophotometer[6] at 400 nm. All samples were assayed in duplicate. The difference in the mineral content of the feed consumed and of the feces excreted was used to calculate the apparent availability of minerals.

Plasma Analysis

On the last day of the experiment, heparinized blood was obtained by cardiac puncture from three randomly selected chickens in each replicate. Plasma was separated immediately by centrifugation of blood for 10 min at 2,000 × *g*. Plasma Ca, Cu, Zn, and total P were determined as indicated in the mineral analyses of the feed and excreta samples.

Bone Analysis

The birds used for blood sampling were killed immediately afterwards by cervical dislocation and the left tibia was removed. After removing any adhering tissue, both ends (heads) were separated from the shaft portion of the tibia for separate mineral analysis. Both head and shaft portions were freeze-dried, fat extracted, and then analyzed for ash minerals (AOAC, 1984) on a fat free dry basis. Bone Ca, Cu, Zn, and P were determined as indicated in the mineral analyses of feed and excreta.

Statistical Analysis

The data were analyzed using the General Linear Models procedure for analysis of variance (SAS Institute, 1985). Significant differences among treatment means were separated by Duncan's new multiple range test (Duncan, 1955) with a 5% level of probability.

TABLE 1. Composition of experimental diets

Ingredients and composition	Corn-soybean diet	Corn-soybean (low-P) diet
	(%)	
Corn, ground	58.0	58.7
Soybean meal	32.1	32.1
Fish meal	3.0	3.0
AV-fat[1]	3.3	3.3
Calcium carbonate	1.8	1.9
Calcium phosphate	0.8	. . .
Salt	0.2	0.2
Vitamin-mineral premix[2]	0.5	0.5
DL-methionine	0.2	0.2
L-lysine·HCl	0.1	0.1
Analyzed composition		
Calcium	1.4	1.3
Total phosphorus	0.7	0.5
Copper, ppm	9.0	7.8
Zinc, ppm	70.9	68.9
Calculated composition		
Crude protein	22.3	22.4
ME, kcal/kg	3,119	3,143
Available phosphorus	0.46	0.33
Lysine	1.3	1.3
Methionine + cystine	0.9	0.9

[1] Animal-vegetable fat blend.

[2] Vitamin-mineral premix supplied the following per kilogram of diet: calcium, 1,000 mg; phosphorus, 450 mg; magnesium, 25 mg; sodium chloride, 550 mg; selenium 0.04 mg; iron, 25 mg; manganese, 22 mg; copper, 1.6 mg; zinc, 16 mg; iodine, 0.14 mg; retinyl acetate, 925 IU; cholecalciferol, 275 IU; dl-α-tocopherol acetate, 5 IU; choline, 40.8 mg; menadione sodium bisulfite, 0.19 mg; riboflavin, 1.9 mg; vitamin B_{12}, 2.2 mg; niacin, 8.1 mg; pantothenic acid, 2.2 mg; biotin, 0.013 mg; folic acid, 0.16 mg; thiamin, 0.5 mg; pyridoxine, 0.9 mg; salinomycin sodium, 7.5 mg.

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[3] Petersime Incubator Co., Gettysburg, OH 45328.

[4] BASF Canada, Inc., Georgetown, ON, Canada, L7G 4R7.

[5] Model 2380, Perkin Elmer, Norwalk, CT 60521.

[6] Model DU-20, Beckman, Fullerton, CA 92713.

RESULTS

Feed Intake, Body Weight, and Feed to Gain Ratio

The effects of phytase supplementation on growth performance are summarized in Table 2. Treatment effect on body weight was significant ($P < 0.05$) at 14 and 21 d. There was a significant effect of sex on body weight at Days 7, 14, and 21. However, interaction between treatment and sex was not significant for any of the growth variables measured. Compared to the normal-P diet, the low-P diet consistently reduced the feed intake as well as the live weight of both male and female chickens throughout the experiment although the difference reached significant levels ($P \leq 0.05$) within each sex only at 21 d. Phytase supplementation of the low-P diet consistently increased the body weight of both male and female chickens compared to those fed the low-P diet; however, the improvement in body weight was significant ($P \leq 0.05$) only at 21 d, when phytase supplementation increased the body weight in male and female chickens by 13.2 and 5.8%, respectively. Dietary phytase supplementation did not affect the efficiency of feed conversion for either male or female chickens throughout the experiment.

Apparent Availability of Minerals

The effects of phytase supplementation on relative retention of minerals are summarized in Table 3. The treatment effects were significant ($P \leq 0.05$) mostly at Day 17, whereas effect of sex and the interaction between treatment and sex were not significant for any of the minerals measured. The low-P diet did not change the relative retention of P. Phytase supplementation of the low-P diet increased ($P \leq 0.05$) the P retention by 12.4 percentage units in males and even though the improvement in P retention in female chickens was not significant, it showed a 6.5 percentage units improvement. The low-P diet reduced ($P \leq 0.05$) the Ca retention by 9.1 percentage units in male chickens at 17 d but the reduction in the females was not significant. Phytase supplementation of the low-P diet increased ($P \leq 0.05$) Ca retention by 12.2 percentage units in males but this increment was not observed in female chickens. The low-P diet significantly reduced ($P \leq 0.05$) the retention of Cu in male chickens but the reduction observed in female chickens was not significant. Phytase supplementation increased ($P \leq 0.05$) the retention of Cu by 19.3 percentage units in male chickens, whereas there was no improvement in female chickens compared to the low-P diet.

There was a significant reduction in the retention of Zn in both male and female chickens fed the low-P diet. Phytase supplementation increased ($P \leq 0.05$) the Zn retention by 62.3 percentage units in male chickens and by 44.3 percentage units in female chickens.

Plasma Minerals

The effects of phytase supplementation on plasma mineral levels are summarized in Table 4. Treatment effect was significant for P and Ca but not for Cu or Zn. The effect of sex on plasma minerals was not significant except for P ($P < 0.04$). The interaction between treatment and sex was not significant for any of the plasma minerals measured.

The low-P diet reduced ($P \leq 0.05$) plasma P in both male and female chickens by 19.1 and 26.3%, respectively. Phytase supplementation increased the plasma P in male and female chickens by 15.7 and 20.7%, respectively and

TABLE 2. The effect of phytase supplementation on feed intake, body weight, and feed to gain ratio of broiler chickens fed corn-soybean diets for 21 d

		0 to 7 d			0 to 14 d			0 to 21 d		
Diet	Sex	Feed intake	Body weight	Feed: gain	Feed intake	Body weight	Feed: gain	Feed intake	Body weight	Feed: gain
		(g)	(g)	(g:g)	(g)	(g)	(g:g)	(g)	(g)	(g:g)
Corn-soybean (Control)	Male	154	143	1.52	477	355	1.53	910^{a}	639^{a}	1.52
Corn-soybean (low-P)		133	130	1.48	394	303	1.49	764^{b}	549^{b}	1.50
Corn-soybean (low-P) + phytase		147	136	1.57	449	332	1.54	867^{ab}	622^{a}	1.49
SEM		9.4	5.7	0.02	28.4	15.2	0.01	30.1	20.5	0.02
Corn-soybean (Control)	Female	135	128	1.60	450	320	1.62	842^{a}	587^{a}	1.54
Corn-soybean (low-P)		124	120	1.57	379	286	1.57	733^{b}	514^{c}	1.55
Corn-soybean (low-P) + phytase		131	118	1.75	397	287	1.63	768^{ab}	544^{b}	1.53
SEM		7.6	6.8	0.04	19.8	8.6	0.03	20.9	4.3	0.02
Corn-soybean (Control)	Male and Female	145	136	1.56	463^{a}	337^{a}	1.57	876^{a}	613^{a}	1.53
Corn-soybean (low-P)		128	125	1.52	387^{b}	294^{b}	1.52	748^{b}	532^{b}	1.52
Corn-soybean (low-P) + phytase		139	127	1.66	423^{ab}	309^{ab}	1.58	817^{ab}	583^{ab}	1.51
SEM		6.6	5.5	0.03	17.3	12.3	0.02	25.5	18.6	0.02
Source of variation		Probabilities								
Treatment		0.231	0.267	0.063	0.054	0.034	0.262	0.007	0.004	0.909
Sex		0.077	0.032	0.025	0.175	0.018	0.037	0.020	0.004	0.432
Treatment × sex		0.826	0.824	0.576	0.756	0.543	0.783	0.474	0.403	0.963

[a–c]Means within columns, within sex classification, with no common superscript differ significantly ($P < 0.05$).

TABLE 3. The effect of phytase supplementation on relative retention of total phosphorus, calcium, copper, and zinc at different ages in broiler chickens fed corn-soybean diets

Diet	Sex	Phosphorus		Calcium		Copper		Zinc	
		10 d	17 d	10 d	17 d	10 d	17 d	10 d	17 d
		(%)							
Corn-soybean (Control)	Male	48.5^{b}	51.0^{b}	34.2	40.7^{a}	3.6	8.5^{a}	16.6^{ab}	8.2^{ab}
Corn-soybean (low-P)		51.3^{ab}	51.0^{b}	36.5	31.7^{b}	−0.8	−24.6^{c}	14.1^{b}	−27.6^{b}
Corn-soybean (low-P) + phytase		57.6^{a}	63.5^{a}	36.7	43.9^{a}	−4.9	−5.4^{b}	41.3^{b}	34.7^{a}
SEM		1.51	1.08	2.83	1.36	5.58	1.96	6.02	8.42
Corn-soybean (Control)	Female	48.7	48.7	33.9	39.8	1.3^{ab}	4.2	9.4	−0.1^{ab}
Corn-soybean (low-P)		53.8	52.7	41.5	36.5	5.1^{a}	−10.0	7.3	−28.3^{b}
Corn-soybean (low-P) + phytase		54.7	59.2	32.2	36.7	−8.4^{b}	−10.7	−0.8	16.1^{a}
SEM		1.81	2.83	3.19	4.39	2.59	6.96	3.48	7.44
Corn-soybean (Control)	Male and Female	48.6^{b}	49.8^{b}	34.1	40.2	2.4	6.3^{a}	13.0	4.0^{b}
Corn-soybean (low-P)		52.6^{a}	51.9^{b}	39.0	34.1	2.2	−17.3^{b}	10.7	−27.9^{c}
Corn-soybean (low-P) + phytase		56.2^{a}	61.3^{a}	34.4	40.3	−6.68	−8.1^{b}	20.3	25.4^{a}
SEM		1.12	1.50	2.07	2.37	2.78	3.99	7.75	5.70
Source of variation		Probabilities							
Treatment		0.011	0.003	0.266	0.170	0.266	0.010	0.209	0.001
Sex		0.934	0.378	0.988	0.691	0.988	0.702	0.003	0.206
Treatment × sex		0.329	0.421	0.356	0.257	0.356	0.171	0.017	0.560

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

the improvement became significant ($P \leq 0.05$) when the values for both sexes were combined. The values for phytase-supplemented birds were comparable with plasma P levels in chickens fed the normal-P diet. The low-P diet significantly increased ($P \leq 0.05$) plasma Ca concentration in both male and female chickens by 34.1 and 22.6%, respectively. Phytase enzyme supplementation reduced ($P \leq 0.05$) plasma Ca for both male and female chickens by 11.5 and 16.7%, respectively. Plasma Cu and Zn were not significantly affected by either the low-P diet or phytase supplementation.

Bone Minerals

The effect of phytase on mineral concentrations in the ash of tibia are summarized in Table 5. The treatment effect on tibia ash content of head and shaft portions was significant ($P \leq 0.05$); however, the effect of sex and interaction of treatment and sex were not significant. The low-P diet significantly reduced ($P \leq 0.05$) the ash content of both the head and the shaft portion of the tibia in both sexes. However, the reduction in ash content was more significant ($P < 0.0004$) in the head portion than in the shaft portion ($P < 0.008$) in both sexes. Mineral concentrations in the ash of the tibia shaft were relatively constant and were not affected by either the low-P diet or phytase supplementation. The effects of treatment, sex, and their interaction on P and Ca concentrations in tibia ash were not significant. Neither the low-P diet nor phytase supplementation caused any change in the concentration of P and Ca in the ash of either portion of tibia for either sex. One exception was that the low-P diet increased ($P \leq$

TABLE 4. The effect of phytase supplementation on concentrations of plasma total phosphorus, calcium, copper, and zinc in 21-d-old broiler chickens fed corn-soybean diets

Diet	Sex	Phosphorus	Calcium	Copper	Zinc
		(mg/dL)		(μg/dL)	
Corn-soybean (Control)	Male	16.4^{a}	10.8^{b}	26	508
Corn-soybean (low-P)		13.3^{b}	14.4^{a}	24	520
Corn-soybean (low-P) + phytase		15.4^{ab}	12.8^{a}	16	436
SEM		0.62	0.42	6.0	40
Corn-soybean (Control)	Female	15.6^{a}	11.5^{b}	21	406
Corn-soybean (low-P)		11.5^{b}	14.1^{a}	18	505
Corn-soybean (low-P) + phytase		13.8^{ab}	11.8^{b}	18	388
SEM		0.63	0.19	5.0	42
Corn-soybean (Control)	Male and Female	16.0^{a}	11.2^{c}	23	457
Corn-soybean (low-P)		12.4^{b}	14.3^{a}	21	512
Corn-soybean (low-P) + phytase		14.6^{a}	12.3^{b}	17	412
SEM		0.55	0.29	3.0	30
Source of variation		Probabilities			
Treatment		0.003	0.0002	0.529	0.140
Sex		0.036	0.503	0.512	0.168
Treatment × sex		0.746	0.081	0.715	0.612

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

TABLE 5. The effect of phytase supplementation of corn-soybean diets on the tibial bone content of ash, total phosphorus, calcium, copper, and zinc in 21-d-old broiler chickens

Diet	Sex	Ash		Phosphorus		Calcium		Copper		Zinc	
		Head	Shaft	Head	Shaft	Head	Shaft	Head	Shaft	Head	Shaft
		(% fat-free tibia DM)		(% of ash)				(ppm in ash)			
Corn-soybean (Control)	Male	34.4^{a}	57.3^{a}	13.8	16.4	30.9^{b}	36.9	12.0^{b}	9.1	445	462
Corn-soybean (low-P)		25.5^{b}	51.4^{b}	13.9	15.2	34.2^{a}	36.8	14.8^{a}	9.0	618	487
Corn-soybean (low-P) + phytase		30.9^{ab}	54.4^{ab}	13.5	14.8	32.6^{ab}	35.6	12.3^{ab}	8.6	488	474
SEM		1.34	1.15	0.19	0.47	0.59	2.28	0.56	0.24	39.1	31.9
Corn-soybean (Control)	Female	33.7^{a}	57.4^{a}	14.5	15.4	38.7	41.9	10.5^{b}	7.0^{b}	388^{b}	392
Corn-soybean (low-P)		23.5^{b}	52.0^{b}	14.2	14.3	38.8	38.4	13.8^{a}	7.9^{a}	576^{a}	460
Corn-soybean (low-P) + phytase		30.3^{a}	55.8^{ab}	13.9	14.6	34.4	35.3	11.6^{ab}	7.8^{a}	436^{ab}	430
SEM		0.83	1.18	0.88	0.66	1.70	2.41	0.67	0.17	36.1	15.1
Corn-soybean (Control)	Male and Female	34.1^{a}	57.4^{a}	14.2	15.9	34.8	39.4	11.3^{b}	8.0	416	427
Corn-soybean (low-P)		24.5^{c}	51.7^{b}	14.1	14.8	36.5	37.6	14.3^{a}	8.5	597	474
Corn-soybean (low-P) + phytase		30.6^{b}	55.1^{a}	13.7	14.7	33.5	35.4	11.9^{b}	8.2	462	452
SEM		0.74	0.73	0.38	0.40	1.69	1.62	0.47	0.43	26.2	20.5
Source of variation		Probabilities									
Treatment		0.0004	0.008	0.766	0.147	0.136	0.303	0.006	0.181	0.007	0.256
Sex		0.262	0.469	0.410	0.203	0.004	0.315	0.092	0.002	0.154	0.063
Treatment × sex		0.805	0.725	0.954	0.744	0.141	0.553	0.789	0.045	0.980	0.705

[a–c]Means within columns, within sex classification, with no common superscript differ significantly ($P < 0.05$).

0.05) the Ca concentration in the ash of the tibia head of male chickens. The treatment effects on the concentrations of Cu and Zn were significant ($P \leq 0.05$) for the ash of the head portion but not for the shaft portion of the tibia. The low-P diet significantly increased ($P \leq 0.05$) Cu concentration in the head by 2.7 percentage units in males and by 3.3 percentage units in females. Phytase supplementation to a low-P diet significantly decreased ($P \leq 0.05$) the Cu concentration in the head portion by 2.5 percentage units in male and by 2.2 percentage units in female chickens. The low-P diet significantly increased ($P \leq 0.05$) Zn concentration in the ash of the tibia head by 188 ppm in female chickens. Phytase supplementation did not significantly decrease Zn concentration in either male or female chickens.

The effects of phytase on the minerals present in the DM of tibia are summarized in Table 6. The treatment effects on the concentrations in tibia DM were significant for P and Ca but not for Cu and Zn. The low-P diet significantly reduced ($P \leq 0.05$) the P content in the tibia head by 1.2 and 1.6 percentage units in male and female chickens, respectively. Neither the low-P diet nor phytase supplementation to a low-P diet had an effect on any mineral content in the DM of the tibia shaft, except that the low-P diet significantly ($P \leq 0.05$) reduced the P content in tibia shaft of male chickens. Phytase supplementation to a low-P diet significantly increased ($P \leq 0.05$) the P content in the tibia head by 0.65 percentage units in male chickens. The low-P diet significantly reduced ($P \leq 0.05$) Ca content in the DM of tibia head by 1.9 and 4.2 percentage units in male and female chickens, respectively. Phytase supplementation to a low-P diet significantly increased ($P \leq 0.05$) Ca content in tibia head by 1.4 percentage units in male chickens but the improvement in female chickens was not significant. Neither a low-P diet nor phytase supplementation to a low-P diet had any effect on the Cu or Zn levels in whole tibia.

DISCUSSION

The results of this study show the effects of microbial phytase supplementation on growth performance, apparent availability of minerals, and plasma and bone mineral contents as indications of nutrient utilization by broiler chickens fed a corn-soybean diet. Phytic acid has been regarded as the primary storage form of both phosphate and inositol in almost all seeds (Cosgrove, 1966). Approximately 66% of the P in corn and 61% of the P in soybean meal is in the form of phytic acid (Nelson *et al.*, 1968). The inability of young broiler chickens to utilize phytic acid has been clearly demonstrated in this study by a slower growth rate (Table 2), a low concentration of plasma P, and reduced bone mineralization observed in chickens fed the low-P diet (Table 5) in which most of the P was provided by corn and soybean meal. Phytase supplementation of the low-P diet yielded a significant improvement in body weight for both sexes at 21 d, compared to chicks fed the low-P diet without phytase supplementation. The body weights achieved by phytase supplementation were comparable to those obtained in the control diet (Table 2), which contained a source of inorganic P to satisfy the requirement (NRC, 1994). The efficacy of phytase on improving body weight was greater for male chickens (13.2%) than for females (5.8%). Similar observations of improved body weight with phytase supplementation have been reported for broiler chickens (Simons *et al.*, 1990; Broz *et al.*, 1994). The improvements in growth

TABLE 6. The effect of phytase enzyme supplementation on the DM percentage of total phosphorus, calcium, copper, and zinc present in the head and shaft portion of tibia of 21-d-old broiler chickens fed corn-soybean diets

		Phosphorus		Calcium		Copper		Zinc	
Diet	Sex	Head	Shaft	Head	Shaft	Head	Shaft	Head	Shaft
		(% in DM)				(ppm in DM)			
Corn-soybean (Control)	Male	5.4^{a}	10.1^{a}	10.6^{a}	21.2	4.1	5.2	153	265
Corn-soybean (low-P)		4.2^{c}	8.5^{b}	8.7^{b}	18.9	3.8	4.7	153	250
Corn-soybean (low-P) + phytase		4.9^{b}	8.7^{b}	10.1^{a}	19.3	3.8	4.7	151	257
SEM		0.09	0.26	0.20	1.30	0.19	0.14	9.0	17.3
Corn-soybean (Control)	Female	5.7^{a}	9.5	12.8^{a}	23.8	3.6	4.0	131	225
Corn-soybean (low-P)		4.1^{b}	8.2	8.6^{b}	19.4	3.3	4.1	135	240
Corn-soybean (low-P) + phytase		4.9^{ab}	8.9	9.9^{ab}	19.3	3.5	4.4	132	240
SEM		0.23	0.34	0.65	1.32	0.19	0.09	11.9	8.8
Corn-soybean (Control)	Male and Female	5.5^{a}	9.8^{a}	11.7^{a}	22.5^{a}	3.9	4.6	142	245
Corn-soybean (low-P)		4.1^{c}	8.3^{b}	8.6^{b}	19.2^{b}	3.5	4.4	144	245
Corn-soybean (low-P) + phytase		4.9^{b}	8.8^{b}	10.0^{b}	19.3^{b}	3.7	4.5	142	249
SEM		0.11	0.21	0.45	0.88	0.18	0.24	8.3	10.8
Source of variation		Probabilities							
Treatment		0.0007	0.007	0.002	0.075	0.273	0.235	0.960	0.951
Sex		0.825	0.331	0.196	0.373	0.024	0.0004	0.063	0.091
Treatment × sex		0.583	0.537	0.083	0.579	0.711	0.026	0.973	0.585

[a–c]Means within columns, within sex classification, with no common superscript differ significantly ($P < 0.05$).

performance observed in the chickens fed phytase may be due to: 1) the release of minerals from the phytate-mineral complex and 2) the utilization of inositol by animals, as suggested by Simons *et al.* (1990) or 3) increased starch digestibility, as suggested by Knuckles and Betschart (1987) or 4) increased availability of protein. Phytate also complexes with proteins, making them less soluble (Smith and Rackis, 1957). It has been shown that phytate-protein complexes are less subject to proteolytic digestion than the same protein alone (Hill and Tyler, 1954). It may be possible that phytase liberates proteins from the complex, making them more available to the animal. However, further investigations are needed to determine the effect of phytase on availabilities of starch, protein, and inositol. Phytase supplementation overcame the depression of growth rate observed on the low-P diet. As a result of the simultaneous increase in both body weight and feed intake, no significant differences in feed to gain ratio were observed. Our findings might be the result of a combination of improvement in nutrient utilization not only of minerals but also of energy and protein. These results agree with the findings of Simons *et al.* (1990) and Perney *et al.* (1993), who did not find any significant improvements in feed conversion of broiler chickens fed a corn-soybean diet supplemented with phytase.

As expected, phytase supplementation increased the relative retention of P by 12.5 percentage units in male chickens (Table 3), which agrees with results of previous studies dealing with chickens (Simons *et al.*, 1990; Broz *et al.*, 1994) and pigs (Young *et al.*, 1993; Lei *et al.*, 1994; Mroz *et al.*, 1994; Bruce and Sundstol, 1995). It is uncertain why female chickens failed to show significant improvement in the relative retention of P (Table 3). Phytase supplementation also improved the relative retention of Ca in male chickens. This improvement was expected because phytase liberates Ca from the Ca-phytate complex and as the availability of P increases, the availability of Ca also increases because both are part of the same complex.

In this experiment, phytase supplementation to the low-P diet significantly improved the relative retention of both Zn and Cu. This result contradicts recent studies in which phytase supplementation to soybean meal did not improve the utilization of Cu (Aoyagi and Baker, 1995) or Zn (Roberson and Edwards, 1994). Phytase supplementation increased the Zn relative retention by almost 62.3 percentage units compared to the low-P diet without phytase supplementation (Table 3). This highly significant improvement in Zn relative retention may be due to higher availability of Zn from the phytate-mineral complex. This observation emphasizes the need to reevaluate the Zn requirement when the broiler diet is supplemented with phytase. Even though the phytase supplementation significantly increased the relative retention of Cu compared to the low-P diet without phytase, it failed to reach the level of Cu relative retention obtained in the normal-P diet. The possible explanation for this observation is that the higher concentration of Zn as the result of phytase activity induces the intestinal synthesis of metallothionein (Blalock *et al.*, 1988), a cysteine-rich metalloprotein, which binds Zn, Cu, and other divalent cations; Cu is much more tenaciously bound to metallothionein than Zn and metallothionein appears to serve primarily as a negative regulator of Cu absorption (Cousins, 1985). L'Abbe and Fischer (1984) have also shown that excess dietary Zn aggravates the signs of low Cu status in rats. We have no obvious explanation why the low-P diet significantly reduced the apparent relative retention of Cu and Zn. It may be possible that the higher content of Ca relative to P in the low-P diet increased the intestinal pH and reduced the soluble fraction of minerals, consequently reducing their availability for absorption (Shafey, 1993).

Plasma P was increased by phytase supplementation to a level comparable to that of the control diet (Table 4) and this result concurs with other studies reported in chickens (Perney *et al.*, 1993; Broz *et al.*, 1994) and pigs (Young *et al.*, 1993). The low-P diet significantly increased the plasma Ca for both male and female chickens. This increase in Ca was expected because a low-P diet normally results in an elevated ionized Ca in the plasma, which depresses the release of parathyroid hormone (PTH), thus reducing the PTH inhibition on tubular reabsorption of phosphate and permitting the urinary excretion of additional Ca absorbed from the gut during low-P diet feeding (Tayler and Dacke, 1984). In contradiction to our observation, some recent studies have shown that phytase had no significant effect on plasma Ca (Edwards, 1993; Roberson and Edwards, 1994). Phytase supplementation did not show any significant effect on plasma Zn (Table 4), in agreement with Roberson and Edwards (1994), who found no significant effect on plasma Zn when a corn-soybean diet supplemented with phytase was fed to broiler chicks; these authors suggested that an adequate level of Zn in the diet might be responsible for the failure to show the significant effect on plasma Zn. Phytase supplementation likewise did not affect the plasma Cu (Table 4).

The percentage of tibia ash was significantly improved by addition of dietary phytase (Table 5), an observation that agrees with the previous studies dealing with chickens (Nelson, 1971; Perney *et al.*, 1993; Broz *et al.*, 1994) and pigs (Young *et al.*, 1993). The improvement in ash percentage in tibia is a good indication of increased bone mineralization due to the fact that there is an increased availability of P, Ca, Zn, and Cu from the phytate-mineral complex by the action of phytase. The shaft portion of the bone represents the more rigid state of the bone, whereas the head is a more active state of change and consequently more susceptible to variation due to availability of minerals. The concentrations of all minerals measured were higher in the shaft portion than in the head except for Cu, which was more concentrated in the head (Table 5). Either low-P or phytase supplementation did not affect the concentration of P, Ca, Cu, and Zn in whole tibia ash (both head and shaft portions); however, phytase supplementation significantly improved the content of P and Ca in the DM of tibia head. In agreement with our observation, Broz *et al.* (1994) showed that phytase supplementation of a corn-soybean diet increased the tibia ash percentage in chickens. However, they could not find any significant difference in P and Ca concentration in tibia ash of broiler chickens. In a recent study with pigs, Young *et al.* (1993) found that phytase supplementation increased bone weight, ash percentage, weight of ash, and weight of Ca and P in the dry fat-free third metatarsal bone but the concentration of P in the ash had not been affected by phytase supplementation. The ash, Cu, and Zn of the head of the tibia bone was significantly affected by the dietary treatment whereas in the shaft portion only the ash content was affected. Dietary treatments had an effect on Ca and P levels in the tibia head DM but not in that of Cu and Zn.

Dietary microbial phytase supplementation to a low-P corn-soybean diet improved the growth, feed consumption, apparent availability of Ca, P, Cu, and Zn, plasma P, percentage of ash, and content of Ca and P in the DM of tibia head of broiler chickens. The efficacy of phytase, particularly in stimulating growth, is higher in male than female chickens. The results obtained in this study clearly indicate the importance of reevaluating mineral requirements, particularly Zn, of broiler chickens when the diet is supplemented with phytase enzyme. This study shows that microbial phytase not only reduces the need for inorganic P but also serves to reduce the need for some other minerals in the diet.

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