Evaluation of the Efficacy of Crude Phytase Prerarations in Broiler Chickens[a]

I. K. Paik*, J. S. Um, S. J. Lee and J. G. Lee[1]
Department of Animal Science and Technology, Chung-Ang University, Ansung-Si, Kyonggi-Do 456-756, Korea

ABSTRACT : An experiment was conducted with day-old 300 commercial male broiler chicks (Arbor Acres®) to evaluate the efficacy of crude phytase prepareations produced from a culture of *Aspergillus ficcum*. The experiment consisted of five dietary treatments; T1, corn-soy control diet with 0.45% non-phytate phosphorus (NPP) for starter period and 0.35% NPP for grower period; T2, control - 0.1% NPP; T3, control - 0.2% NPP; T4, T3+600 U of crude phytase (broth+cell); and T5, T3+600 U of crude phytase (broth). The body weight gain, feed intake, and feed/gain of chickens fed T1 diet was highest ($p<0.01$) among treatments. BW gain and feed intake of T4 and T5 were greater than those of T3 but were less than those of T1 and T2. T3 was highest in mortality among treatments. Decreasing the NPP level lowered availability of DM, crude ash, ether extract, crude fiber, Zn, and Fe but supplementation of crude phytase preparations improved the availability of these nutrients as well as those of Ca, P and Cu. Excretion of P and Cu significantly decreased as the NPP level in the diet decreased. Further reduction of P and Cu excretion and reduction of Ca, Mg and Fe excretion were achieved by supplementation of crude phytase preparations. The serum concentrations of Ca, P, Mg, Zn, Fe, and Cu were significantly increased by crude phytase supplementation. The weight and length of tibia, and contents of crude ash, Ca, P, Mg, and Zn were adversely affected by lowering NPP level but partially recovered by supplementation of crude phytase preparations. In conclusion, lowering NPP level in the broiler diet significantly depressed the performance. Supplementation of crude phytase preparations produced from *Aspergillus ficuum* could partially recover the depression. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 5 : 673-680*)

Key Words : Crude Phytase, *Aspergillus ficuum*, Non-Phytate Phosphorus, Broiler Performance, Serum, Tibia

INTRODUCTION

Phytic acid, myo-inositol hexakisphosphate, is the major storage form of phosphate in cereals and oilseeds. Phytic acid can form insoluble salt by combining with di- and tri-valent cations such as Ca, Mg, Fe, Zn, and Cu (Oberleas, 1973; Erdmann, 1979; Morris, 1986; Reddy et al., 1982). Thus, the fact that phytate lowers the nutritional value of food and feed by binding to proteins and metal ions has led to the search for suitable enzyme hydrolyzing phytate to make it more favorable to monogastric animals (Howson and Davis, 1983). The phytate-hydrolyzing enzyme, phytase, can improve feed quality by hydrolyzing phytic acid to inorganic phosphate and inositol mono- to penta-phosphates (Gibson, 1987).

Phytase occurs in plant and microorganisms. For plant phytase, the optimum pH is about 5.0 (Hill and Tyler, 1954). It has been reported that phytase (EC 3.1.3.26) from plant origin fails to have the activity at low pH while microbial phytase (EC 3.1.3.8) is active over a wide pH range (Simons et al., 1990). Besides pH or temperature, the efficacy of dietary phytase is known to depend on the contents and interactions of various nutrients, especially minerals such as multivalent metal cations. Among the animals, birds are especially sensitive to dietary P because they have the characteristics of low P storage and fast growth (Kornegay et al., 1996). Dietary calcium, zinc or iron also affect the phytate utilization (Mitchell and Edwards, 1996; Larsson et al., 1996). Schoner et al. (1993) reported that the apparent retention or total amount of retention of P and Ca by broilers might be sensitive indicators for evaluating P availability. The objective of this study is to evaluate the effects of crude phytase preparations produced from *Asspergillus ficuum* in supplementating to corn-soybean basal diets in broilers.

MATERIALS AND METHODS

Experimental diet

The formula and chemical composition of experimental diets are shown in table 1 and 2. Diets were formulated on isocaloric and isonitrogenous basis, and to include different levels of tricalcium phosphate (TCP) as a source of non-phytate phosphorus (NPP). The levels of NPP were lowered by 0.1% and 0.2% from corn-soy control diet (T1) by decreasing the TCP level by 0.55% and 1.11% (T2 and T3), respectively. Two types of crude phytase, broth type (liquid enzyme produced from a culture of *Aspergillus ficuum*) and

* Address reprint request to I. K. Paik. Tel: +82-334-670-3028, Fax: +82-334-676-2196, E-mail: ikpaik@cau.ac.kr.
[1] Dept. of Biotechnology, Chung-Ang University.
[a] This research was financially supported by the KMAF-SGRP (Korean Ministry of Agriculture and Forestry - Special Grants Research Program) and Easy Bio System, Inc., Korea.
Received August 24, 1999; Accepted December 9, 1999

Table 1. Formula and composition of broiler starter diets

Ingredients	Treatments[3]				
	T1	T2	T3	T4	T5
	(%)				
Corn	58.41	58.45	58.49	58.49	58.49
Soybean meal (44% CP)	24.27	24.43	24.59	24.59	24.59
Corn gluten meal	11.79	11.67	11.56	11.56	11.56
Tricalcium phosphate (18% P)	1.79	1.24	0.68	0.68	0.68
Animal fat	1.50	1.50	1.50	1.50	1.50
Limestone	0.77	1.25	1.73	1.73	1.73
Broiler premix[1]	0.73	0.73	0.73	0.73	0.73
Salt	0.40	0.40	0.40	0.40	0.40
Lysine-HCl (78%)	0.19	0.19	0.18	0.18	0.18
DL-Methionine (50%)	0.15	0.15	0.15	0.15	0.15
Crude phytase[2]	-	-	-	+	+
Total	100.00	100.00	100.00	100.00	100.00
Caluclated composition					
ME, kcal/kg	3100	3100	3100	3100	3100
Crude protein	23.0	23.0	23.0	23.0	23.0
Lysine	1.10	1.10	1.10	1.10	1.10
Methionine+Cystine	0.90	0.90	0.09	0.09	0.09
Calcium	1.00	1.00	1.00	1.00	1.00
Non-phytate phosphorus	0.45	0.35	0.25	0.25	0.25
Total phosphorus	0.71	0.61	0.51	0.51	0.51

[1] Provides per kg of diet: vitamin A, 16,000 IU; vitamin D_3, 3,200 IU; vitamin E, 30 IU; vitamin K_3, 6.5 mg; vitamin B_1, 2.6 mg; vitamin B_2, 10.4 mg; vitamin B_6, 6.5 mg; vitamin B_{12}, 39 μg; folic acid, 1.3 mg; niacin, 52 mg; pantothenic acid, 19.5 mg; I, 0.5 mg; Zn, 50 mg; Mn, 70 mg; Fe, 80 mg; Cu, 10 mg; Se, 0.4 mg.

[2] Crude phytase produced from a culture of *Aspergillus ficcum* at Chung-Ang University.

[3] T1=control diet, T2=control-0.1% NPP, T3=control-0.2% NPP, T4=T3+600 U of crude phytase (broth+cell)/kg of diet, T5=T3+600 U of crude phytase (broth)/kg of diet.

broth+cell type (mixed slurry of culture and microbial cell) were tested in this experiment by adding to the low NPP diet (T3). These crude phytase preparations added to diets were produced by modifying the method of Gibson (1987). Phytase activity in broth+cell type and broth type were 22 and 23 U/mL, respectively. One unit of phytase activity was described as the quantity of enzyme that liberates 1 μmol inorganic P/min from 1.5 mM-sodium phytate at pH 5.5 and 55C (Harland and Harland, 1980).

Feeding regimen

Day-old 300 Arbor Acres male broiler chicks were randomly allotted to 25 cages of 12 birds each. Five cages were assigned to each of the fallowing five dietary treatments: T1, control diet (starter: 0.45% NPP and grower: 0.35% NPP); T2, control - 0.1% NPP; T3, control - 0.2% NPP; T4, T3+600 U of crude phytase (broth+cell)/kg of diet; and T5, T3+600 U of crude phytase (broth)/kg of diet. Diets were offered in mash form, and feed and water were given ad libitum during 35 d feeding periods (starter: 1-21d, grower: 21-35d). All birds were housed in wire-floored battery cages and exposed to continuous lighting.

Performance, nutrients availability, and excretion

Weight gain and feed consumption were measured weekly and mortality was recorded daily. At the end of the feeding trial, 6 birds from each treatment were selected and randomly assigned to metabolic cages to assess the availability and excretion of nutrients of experimental diets. Excreta of chickens were collected for 3 d. Foreign substances (feathers, scurfs, etc.) intermixed within the collected excreta were eliminated before drying at 60℃ for 48 h and subsequent grinding. Feeds and excreta were analyzed by chemical procedures (AOAC, 1990) for proximate compositions. The availability of nutrients was calculated by dividing the amount of retained nutrient (ingested nutrient-excreted nutrient) with the amount of ingested nutrient. The excretion is represented by the amount of nutrient output through feces per bird per day. To determine the concentration of Ca, P, Mg, Zn, Fe, and Cu, samples of feeds and feces were dry ashed (AOAC, 1990) and assayed at specific wavelengths of each element (Ca, 317.933; P, 214.914; Mg, 279.079; Fe, 259.940; Zn, 213.856; and Cu, 324.754 nm) using ICP (Inductively Coupled Plasma) Emission Spectrometer (Model JY, Jobin Yvon, Longiumeau, Cedex 91165,

Table 2. Formula and composition of broiler grower diets

Ingredients	Treatments[3]				
	T1	T2	T3	T4	T5
	(%)				
Corn	65.33	65.37	65.40	65.40	65.40
Soybean meal (44% CP)	23.06	23.22	23.38	23.38	23.38
Corn gluten meal	6.82	6.71	6.59	6.59	6.59
Tricalcium phosphate (18% P)	1.26	0.71	0.15	0.15	0.15
Animal fat	1.50	1.50	1.50	1.50	1.50
Limestone	0.98	1.46	1.73	1.73	1.73
Broiler premix[1]	0.58	0.58	0.58	0.58	0.58
Salt	0.27	0.27	0.27	0.27	0.27
Lysine-HCl (78%)	0.15	0.15	0.15	0.15	0.15
DL-Methionine (50%)	0.04	0.04	0.04	0.04	0.04
Crude phytase[2]	-	-	-	+	+
Total	100.00	100.00	100.00	100.00	100.00
Caluclated composition					
ME, kcal/kg	3100	3100	3100	3100	3100
Crude protein	20.0	20.0	20.0	20.0	20.0
Lysine	1.00	1.00	1.00	1.00	1.00
Methionine+Cystine	0.72	0.90	0.72	0.72	0.72
Calcium	0.90	0.72	0.90	0.90	0.90
Non-phytate phosphorus	0.35	0.25	0.15	0.15	0.15
Total phosphorus	0.61	0.51	0.41	0.41	0.41

[1] Provides per kg of diet: vitamin A, 12,000 IU; vitamin D_3, 2,400 IU; vitamin E, 20 IU; vitamin K_3, 5 mg; vitamin B_1, 2 mg; vitamin B_2, 8 mg; vitamin B_6, 5 mg; vitamin B_{12}, 30 μg; folic acid, 1 mg; niacin, 40 mg; pantothenic acid, 15 mg; I, 0.5 mg; Zn, 50 mg; Mn, 70 mg; Fe, 80 mg; Cu, 10 mg; Se, 0.4 mg.

[2] Crude phytase produced from a culture of *Aspergillus ficcum* at Chung-Ang University.

[3] T1=control diet, T2=control-0.1% NPP, T3=control-0.2% NPP, T4=T3+600 U of crude phytase (broth+cell)/kg of diet, T5=T3+600 U of crude phytase (broth)/kg of diet.

France).

Serum analysis

After the feeding trial, twelve chickens were randomly selected from each treatment, and sacrificed by cervical dislocation. Then blood samples were collected by cardiac puncture in non-heparinized test tubes. Blood samples were allowed to clot at room temperature for several hours. Sera obtained were separated by centrifugation for 15 min at 2,000×g. Serum minerals were determined using ICP Emission Spectrometer after wet washing with HNO_3 (AOAC, 1990).

Tibial bone analysis

Left tibias were removed from killed birds and dried for 72 hours at 60℃. After drying, tibial weight, length, and girth were measured followed by grinding and fat extraction. The fat-free dry tibias were analyzed for ash and minerals. Tibial minerals were assayed by the same procedures as feed and excreta.

Statistical analysis

The data obtained from the experiments were analyzed by completely randomized design using General Linear Models (GLM) procedures (SAS Institute, 1985). Differences between means of each treatment were determined at $p<0.05$ using the Duncan's new multiple range test (Duncan, 1955). Least Square Means (LSM) option was also used to include body weight at death as a covariate in statistical analyses of weight, length, and girth of tibia.

RESULTS

The weight gain, feed intake, feed/gain, and mortality are shown in table 3. All the parameters measured were significantly ($p<0.05$) affected by the treatments, that is, the level of NPP and supplementation of crude phytase preparations. The BW gain and feed intake decreased, and feed conversion ratio (feed/gain) and mortality increased as the level of NPP decreased. Supplementation of crude phytase preparations (T4 and T5) to the low NPP diet (T3) significantly improved BW gain and feed intake but BW gain did not reach those of T1 and T2. There was no improvement in feed/gain by supplementing crude phytase preparations. Mortality

Table 3. Weight gain, feed intake, feed/gain, and mortality in broiler chickens fed experimental diets

Item	Treatments[1]					SEM
	T1	T2	T3	T4	T5	
Weight gain, g/bird						
1-21 d	612.7^{a}	534.2^{b}	384.1^{d}	479.6^{bc}	646.5^{c}	18.83
22-35 d	606.1^{a}	555.4^{ab}	352.7^{c}	521.8^{b}	496.7^{b}	22.35
1-35 d	1218.8^{a}	1089.5^{b}	736.8^{d}	1001.4^{c}	961.3^{c}	25.79
Feed intake, g/bird						
1-21 d	849.0^{a}	747.5^{b}	541.6^{c}	699.7^{b}	684.6^{b}	23.67
22-35 d	1118.1^{a}	1007.9^{b}	744.5^{c}	1058.7^{ab}	1005.4^{b}	33.55
1-35 d	1967.1^{a}	1755.5^{b}	1286.1^{c}	1758.4^{b}	1680.0^{b}	43.55
Feed/gain (g/g)						
1-21 d	1.38^{c}	1.40^{bc}	1.41^{abc}	1.46^{ab}	1.48^{a}	0.02
22-35 d	1.85^{b}	1.82^{b}	2.12^{a}	2.04^{a}	2.04^{a}	0.05
1-35 d	1.61^{b}	1.62^{b}	1.75^{a}	1.76^{a}	1.76^{a}	0.02
Mortality, %						
1-21 d	3.3^{b}	6.7^{b}	26.7^{a}	11.7^{b}	10.0^{b}	4.92
22-35 d	6.8	7.3	12.4	13.3	5.3	3.16
1-35 d	10.0^{b}	13.3^{b}	36.7^{a}	23.3^{ab}	15.0^{b}	4.62

[1] T1=control diet with non-phytate phosphorus (NPP) level of 0.45% (starter) and 0.35% (grower), T2=T1-0.1% NPP, T3=T1-0.2% NPP, T4=T3+600 U of crude phytase (broth+cell)/kg of diet, T5=T3+600 U of crude phytase (broth)/kg of diet.

[a,b,c,d] Values with different superscripts in the same row are significantly different ($p<0.05$).

Table 4. Availability of nutrients in diets fed to broiler chickens

Item	Treatments[1]					SEM
	T1	T2	T3	T4	T5	
	(%)					
DM	82.1^{a}	80.4^{ab}	78.2^{b}	80.6^{ab}	79.7^{ab}	0.89
Crude ash	46.7^{a}	38.2^{b}	31.2^{b}	47.0^{a}	34.1^{b}	2.62
Crude protein	78.2	76.2	74.3	73.5	76.0	2.43
Crude fat	88.8^{a}	85.1^{ab}	80.6^{c}	86.4^{ab}	83.8^{bc}	1.41
Crude fiber	26.0^{a}	19.3^{ab}	14.5^{b}	18.9^{ab}	19.7^{ab}	3.29
NFE	90.3	90.0	89.6	91.3	90.1	0.73
Calcium	40.0^{bc}	35.1^{c}	34.4^{c}	56.1^{a}	44.9^{b}	3.05
Phosphorus	44.2^{bc}	37.8^{c}	40.6^{bc}	58.4^{a}	48.0^{b}	2.28
Magnesium	31.1	30.9	28.7	37.7	27.5	3.21
Sinc	30.4^{a}	32.0^{ab}	22.8^{b}	42.1^{a}	42.4^{a}	3.58
Iron	24.2^{ab}	28.9^{ab}	14.4^{b}	28.6^{ab}	37.4^{a}	4.32
Copper	3.9^{b}	9.5^{b}	26.1^{a}	41.4^{a}	25.9^{a}	4.04

[1] T1=control diet with non-phytate phosphorus (NPP) level of 0.45% (starter) and 0.35% (grower), T2=T1-0.1% NPP, T3=T1-0.2% NPP, T4=T3+600 U of crude phytase (broth+cell)/kg of diet, T5=T3+600 U of crude phytase (broth)/kg of diet.

[a,b,c] Values with different superscripts in the same row are significantly different ($p<0.05$).

was very high (36.7%) in T3 and supplementation of crude phytase preparations reduced mortality. There were no significant differences in measured parameters between crude phytase prepared with broth plus cell and that with broth alone.

Availability of nutrients is shown in table 4. Reduction of NPP level significantly decreased availability of DM, crude ash, crude fat, crude fiber, Zn and Fe but increased that of Cu. Supplementation of crude phytase preparations increased availability of crude ash, crude fat, Ca, P, Zn and Fe.

Data on the nutrient excretion are shown in table 5. Reduction of NPP level significantly decreased the excretion of P, Fe and Cu. Supplementation of crude phytase preparations decreased the excretion of Ca, P and Mg.

Table 5. Excretion of nutrients by broiler chickens fed experimental diets

Item	Treatments[1]					SEM
	T1	T2	T3	T4	T5	
	(g/bird/d)					
DM	13.38	14.89	13.11	12.62	12.71	1.00
Crude ash	2.93	3.18	2.96	2.58	2.96	0.23
Nitrogen	0.72	0.75	0.71	0.70	0.68	0.067
NEF	4.66	5.63	4.57	4.66	4.58	0.54
Calcium	0.47^{a}	0.50^{a}	0.44^{ab}	0.35^{b}	0.40^{ab}	0.04
Phosphorus	0.28^{a}	0.23^{b}	0.14^{c}	0.10^{c}	0.12^{c}	0.014
Magnesium	0.097^{ab}	0.102^{a}	0.084^{ab}	0.079^{b}	0.082^{ab}	0.007
	(mg/bird/d)					
Zinc	6.79	6.78	6.10	5.45	5.43	0.52
Iron	15.74^{a}	14.85^{a}	11.66^{ab}	11.45^{ab}	9.91^{b}	1.45
Copper	1.11^{a}	1.00^{a}	0.68^{b}	0.68^{b}	0.70^{b}	0.076

[1] T1=control diet with non-phytate phosphorus (NPP) level of 0.45% (starter) and 0.35% (grower), T2=T1-0.1% NPP, T3=T1-0.2% NPP, T4=T3+600 U of crude phytase (broth+cell)/kg of diet, T5=T3+600 U of crude phytase (broth)/kg of diet.

[a,b,c] Values with different superscripts in the same row are significantly different ($p<0.05$).

Table 6. Serum Ca, P, Mg, Zn, Fe and Cu in broiler chickens fed experimental diets

Item	Treatments[1]					SEM
	T1	T2	T3	T4	T5	
	(mg/dL)					
Calcium	13.57^{c}	14.09^{c}	14.48^{c}	24.58^{a}	20.25^{b}	0.90
Phosphorus	18.92^{a}	15.43^{b}	13.43^{b}	18.23^{a}	15.74^{b}	0.81
Magnesium	4.42^{c}	4.25^{c}	3.67^{d}	5.49^{a}	4.83^{b}	0.14
Iron	0.53^{c}	0.67^{bc}	0.86^{b}	1.22^{a}	1.32^{a}	0.09
	(μg/dL)					
Zinc	255^{c}	251^{c}	270^{c}	353^{b}	487^{a}	28.0
Copper	18^{b}	19^{b}	16^{b}	32^{a}	30^{a}	2.00

[1] T1=control diet with non-phytate phosphorus (NPP) level of 0.45% (starter) and 0.35% (grower), T2=T1-0.1% NPP, T3=T1-0.2% NPP, T4=T3+600 U of crude phytase (broth+cell)/kg of diet, T5=T3+600 U of crude phytase (broth)/kg of diet.TABLE 6. Serum Ca, P, Mg, Zn, Fe and Cu in broiler chickens fed experimental diets.

[a,b,c,d] Values with different superscripts in the same row are significantly different ($p<0.05$).

The concentrations of serum minerals are shown in table 6. Reduction of NPP level significantly decreased serum levels of P and Mg, and increased that of Fe. Supplementation of crude phytase preparations significantly increased serum levels of Ca, P, Mg, Fe, Zn and Cu.

Table 7 showes weight, length and girth of dry tibia, and ash content and mineral composition of fat-free dry tibia. The weight, length and girth of tibia decreased as the level of NPP in the diets decreased. Supplementation of crude phytase preparations only partially alleviated the decrease. The content of tibial crude ash, Ca, P, Mg and Zn significantly decreased and that of Fe increased as the level of NPP decreased. The supplementation of crude phytase preparations significantly increased tibial crude ash, Ca, P and Mg. Tibial content of Ca was greater in T4 than T5 and that of Fe was greater in T5 than T4.

DISCUSSION

The supplementation of crude phytase produced from a culture of *Aspergillus ficcum* led to the improvement in growth performance in this experiment. The BW gains of broilers given the diets containing low level of P (0.2% less NPP level than the normal NPP level of the control diet) with 600 U of crude phytase/kg of diet (T4 and T5) were significantly improved when compared to the diet containing no

Table 7. Weight, length and girth, and the contents of ash and minerals of tibia of broiler chickens fed experimental diets

Item	Treatments[1]					SEM
	T1	T2	T3	T4	T5	
Weight[2], g	4.15[a]	3.78[b]	2.85[d]	3.21[c]	3.17[c]	0.14
Length[2], cm	8.43[a]	8.12[b]	7.26[d]	7.64[c]	7.59[c]	0.13
Girth[2], cm	2.25[ab]	2.33[a]	2.15[b]	2.26[ab]	2.19[ab]	0.08
	(% of fat-free dry tibia)					
Crude ash	58.72[a]	50.14[c]	44.09[d]	52.17[bc]	54.86[b]	0.96
Calcium	19.71[a]	16.93[b]	12.70[d]	14.59[c]	13.53[d]	0.51
Phosphorus	9.87[a]	8.23[b]	6.30[d]	7.17[c]	6.75[cd]	0.27
Magnesium	0.45[a]	0.93[b]	0.33[c]	0.38[b]	0.38[b]	0.017
	(μg/g of fat-free dry tibia)					
Zinc	409.9[a]	316.0[b]	350.4[b]	386.7[ab]	336.4[b]	18.15
Iron	324.9[b]	329.3[b]	402.1[a]	332.0[b]	412.8[a]	30.45
Copper	5.4	5.6	5.3	5.5	5.4	0.44

[1] T1=control diet with non-phytate phosphorus (NPP) level of 0.45% (starter) and 0.35% (grower), T2=T1-0.1% NPP, T3=T1-0.2% NPP, T4=T3+600 U of crude phytase (broth+cell)/kg of diet, T5=T3+600 U of crude phytase (broth)/kg of diet.

[2] Dry tibia and data presented as least square means with body weight at death used as covariate.

[a,b,c,d] Values with different superscripts in the same row are significantly different ($p<0.05$).

phytase. However, it failed to reach the BW gain of T1 or T2.

During the starter period, decreasing the NPP level from 0.45% to 0.35% and 0.25% led to depression of BW gain by 12.8 and 37.3%, respectively. Crude phytase treatments (T4 and T5) at 0.25% NPP level increased the BW gains by 25% and 21%, respectively, compared to that of T3. These results are similar to the findings of Perney et al. (1993) who reported that weight gain was depressed by 48% by decreasing dietary available P (P_{av}) from 0.45 to 0.21%, while decreasing dietary P_{av} from 0.44% to 0.32% decreased weight gain by only 12%. Schoner et al. (1993) also found that the phytase supplementation improved the growth rate, feed intake, and P and Ca retention when broilers were fed diets containing a constantly low level of P (0.35% total P). In a subsequent broiler experiment in our laboratory, NPP level of corn-soy broiler diets could be safely lowered by 0.2% by supplementing 600 U of a commercial microbial phytase. Compared to this result, the present result indicates that lowering NPP level by 0.2% is not safe with supplementation of 600 U of crude phytase preparations. We do not have resonable explanation why the efficacy of the crude phytase preparations is different from that of a commercial microbial phytase at the same supplementation unit (600 U/kg diet). It is possible that crude phytase may contain other bioactive substances besides phytase.

The mortality was about 37% in broilers fed the diet containing 0.25% NPP without crude phytase (T3) indicating that proper phosphorus levels in the diets are critical to the livability of broilers. This result is similar to the findings by Denbow et al. (1995) who reported 45% mortality of broilers fed 0.20% NPP diet without phytase. In the present experiment, crude phytase supplementation reduced mortality.

Even though the crude phytase supplementation did not improve the growth performance as much as we expected in the first place, there was no doubt that it improved the phytate P utilization. Compared to the low P diet without phytase supplementation (T3), several nutrients such as crude ash, ether extract, Ca, P, Zn and Fe were better utilized by the supplementation of crude phytase preparations. Improved nutrient availability by crude phytase supplementation along with low dietary NPP reduced excretion of Ca, P, Mg, Fe and Cu. Reduced excretion of such minerals may be desirable to the environment.

Supplemental microbial phytase has been shown to be very effective for improving dietary phytate P bioavailability (Simons et al., 1990; Yi et al., 1994a, b; Denbow et al., 1995). In a study with turkey poults, Sanders et al. (1992) reported that birds showed the highest percentage of phytate P retained and probably rate of phytate hydrolysis when fed diets containing low dietary levels of NPP. Results from this experiment have shown that phytase supplementation to low P diet (0.2% NPP reduction from normal NPP) can alter the quantitative characteristics of tibia. The tibias from birds fed P-deprived diets were shorter and had reduced mass and mineral contents. Edwards and Veltmann (1983) and Yoshida (1986) reported similar findings. In the present experiment, the phytase supplementation increased the tibia ash compared to the low P diets without phytase, although it failed to reach the level acquired in the control of normal NPP level. The

increased content of tibia ash suggests an improvement in bone mineralization due to increased P and Ca utilization. As Perney et al. (1993) claimed, the liberation of inorganic P and Ca from the phytate molecule by the phytase enzyme in the digestive tract may cause the increased utilization of P and Ca. Microbial phytase supplementation increased the ash percentage of toes and tibias because it affected P and Ca utilization, which were responsible for more than 50% of the ash (Qian et al., 1996).

As can be seen in table 6, lowering NPP level decreased serum concentrations of P and Mg while crude phytase supplementation increased those of Zn and Cu as well as Ca, P, Mg and Fe. It is interesting to note that serum Fe level increased as the level of dietary NPP decreased, to which tibial Fe level responded accordingly. Supplementation of crude phytase preparations further increased serum Fe but failed to further increase tibial Fe. A few studies indicated that supplemental microbial phytase improved the bioavailability and absorption or retention of Zn in broiler and pigs (Lei et al., 1993; Yi et al., 1996a). It has been also known that an antagenistic interaction exist between Zn and Cu (Aoyagi and Baker, 1995). Sebastian et al. (1996a) reported that the supplementation of phytase significantly increased the relative retention of Cu compared to the low P diet without phytase. However, they failed to observe relative retention of Cu to reach the level obtained in the normal P diet. The higher concentration of Zn as the result of phytase activity induces the intestinal synthesis of metallothionein (Blalock et al., 1988), a cystein-rich metalloprotein, which binds Zn, Cu, and other divalent cations (Shafey, 1991). Cu is much more tenaciously bound to metallothionein than Zn, thus metallothionein appears to serve primarily as a negative regulator of Cu absorption (Cousins, 1985). Roberson and Edward (1994) reported that adding phytase increased tibia Zn concentration but did not improve apparent Zn retention in broiler chicks. Biehl et al. (1995) found that the addition of 1,200 U of phytase/kg of diet to a glucose-soybean concentrate diet (13 ppm of Zn) increased growth rate by 40% and total tibia Zn by 107% in chicks. Sebastian et al. (1996b) found that the low P diet significantly reduced the apparent relative retention of Cu and Zn. They explained 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

Mineral interactions are complex. Present study showed that lowering dietary NPP level decreased the availability of Ca, Zn and Fe while increasing that of Cu. Supplementation of crude phytase preparations improved availability of all these minerals. Nutrient excretion, serum concentration and tibial mineral contents did not precisely respond to the availability of minerals in each treatment but positive effects of supplementary crude phytase preparations were consistant throughout all the parameters measured. Generally, crude phytase with cell+broth showed better response than that with broth alone.

It can be concluded that, the crude phytase supplementation to broiler diets containing a very low level of NPP, 0.2% less NPP or 56% of the normal level, improved the growth performances and bioavailability of nutrients. However, the improvement was not sufficient enough to compensate the negative impact due to low dietary NPP. Further investigations are needed to accurately evaluate the efficacy of the crude phytase preparations.

REFERENCES

Aoyagi, S. and D. Baker. 1995. Effects of microbial phytase and 1,25-dihydroxycholecalciferol on dietary copper utilization in chicks. Poultry Sci. 74:121-126.

Association of Offical Analytical Chemist. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Blalock, T. A., M. A. Dunn and R. J. Cousins. 1988. Metallothionein gen expression in rats: tissue-specific regulation by dietary copper and zinc. J. Nutr. 118:222-228.

Biehl, R. R. and D. H. Baker. 1997. Utilization of phytate and nonphytate phosphorus in chicks as affected by source and amount of vitamin D_3. J. Anim. Sci. 75:2986-2993.

Cousins, R. J. 1985. Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65:238-309.

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

Duncan, D. B. 1955. Multiple range and multiple F test. Biometrics. 11:1-42.

Edwards, H. M. and J. R. Veltmann. 1983. The role of calcium and phosphorus in the etiology of tibial dyscondroplasia in young chicks. J. Nutr. 113:1568-1575.

Erdmann, J. W. 1979. Oil seed phytates; nutritional implications. J. Am. Oil Chem. Soc. 56:736-741.

Gibson, D. M. 1987. Production of extracellular phytase from *Aspergillus ficuum* on starch media. Biotechnology Letters. 9(5):305-310.

Harland, B. F. and J. Harland. 1980. Fermentative Reduction of phytate in rye, white, and whole wheat breads. Cereal Chem. 57(3):226-229.

Hill, R. and C. Tyler. 1954c. The reaction between phytate and protein. J. Agric. Sci. 44:324-328.

Howson, S. J. and R. P. Davis. 1983. Production of phytate-hydrolyzing enzyme by some fungi. Enz. Microb. Technol. 5:377-382.

Kornegay, E. T., D. M. Denbow, Z. Yi and V. Ravindran. 1996. Responses of broilers to graded levels of microbial

phytase added to maize-soybean-meal-based diets containing three levels of non-phytate phosphorus. Br. J. Nutr. 75:839-852.

Larsson, M., L. Rossand-Hulthen, B. Sandstrom and Ann-Sofie Sandberg. 1996. Improved zince and iron absorption from breakfast meals containing malted oats with reduced phytate content. Br. J. Nutr. 76:677-688.

Lei, X. G., P. K. Ku, E. R. Miller, D. E. Ullrey and M. T. Yokoyama. 1993. Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. J. Nutr. 123:1117-1123.

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

Morris, E. R. 1986. Phytate and dietary mineral bioavailability. In: Phytic Acid: Chemistry and Applications (Ed. E. Graf). Pilatus Press, Minneapolis, MN. pp. 57-56

Oberleas, D. 1973. Phytates. In: Toxicants Occurring Naturally in Foods. 2nd Ed. National Academy Press, Washington, DC, USA. p. 363.

Perney, K. A., A. H. Canton, M. L. Straw and K. L. Herkelman. 1993. The effect of dietary phytase on growth performance and phosphorus utilization of broiler chicks. Poultry Sci. 72:2106-2114.

Qian, H., E. T. Kornegay and D. M. Denbow. 1996. Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium to P ratios and phosphorus levels. Poultry Sci. 75:69-81.

Reddy, N. R., C. V. Balakrishnan and D. K. Salunkhe. 1982. Phytates in legumes. Adv. Food Res. 28:1-92.

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

Sanders, A. M., H. M. Edwards, Jr. and G. N. Rowland III. 1992. Calcium and P requirements of the very young turkey as determined by response surface analysis. Br. J. Nutr. 67:421-435.

SAS Institute. 1985. SAS User's Guide: Statistics (Version 6th Ed.). SAS Institute Inc., Cary, NC.

Sebastian, S., S. P. Touchburn, E. R. Chevez and P. C. Lague. 1996a. 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. Poultry Sci. 75:729-736.

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

Schoner, B. R. J, P. P. Hoppe, G. Schwarz and H. Wiesche. 1993. Effects of microbial phytase and inorganic phosphate in broiler chickens: performance and mineral retention at various calcium levels. J. Anim. Physiol. Anim. Nutr. 69:235-244.

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

Shafey, T. M. 1993. Calcium tolerance of growing chickens: effect of ratio of dietary calcium to available phosphorus. World's Poult. Sci. 49:5-18.

Shafey, T. M., M. W. McDonald and J. G. Dingle. 1991. Effects of dietary calcium and available phosphorus concentration on digesta pH and on the availability of aclaium, iron, magnesium and zinc from the intestinal contents of meat chickens. Br. Poult. Sci. 32:185-194.

Yi, Z., E. T. Kornegay, M. D. Lindemann and V. Ravindran. 1994a. Effectiveness of Natuphos phytase for improving the bioavailabilities of phosphorus and other nutrients in soybean meal-based semi-purified diets for young pigs. J. Anim. Sci. 72(suppl. 2):7(Abstr.)

Yi, Z., E. T. Kornegay, V. Ravindran and D. M. Denbow. 1994b. Improving availability of corn and soybean meal P for broilers using Natuphos phytase and calculation of replacement values of inorganic P by phytase. Poultry Sci. 73(Suppl. 1):89(Abstr.)

Yi, Z., E. T. Kornegay, V. Ravindran and D. M. Denbow. 1996a. Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values of phytase. Poultry Sci. 75:240-249.

Yoshida, M. 1986. Difference in availability of phosphorus in cereals by the chick. Jpn. Poult. Sci. 23:1-8.