The Effects of Phytase Supplementation on the Performance of Broiler Chickens Fed Diets With Different Levels of Non-Phytate Phosphorus**

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ABSTRACT : An experiment was conducted to determine the effects of phytase supplementation to the diets containing different levels of non-phytate phosphorus (NPP). A 3×2 factorial arrangement of treatments was employed. There were three dietary NPP levels of control (C) (0.45% for starter diet and 0.35% for grower diet), C-0.1% NPP (0.35% for starter diet and 0.25% for grower diet), and C-0.2% NPP (0.25% for starter diet and 0.15% for grower diet) and two phytase levels (0 and 500 U/kg). Reduced dietary NPP decreased feed intake and weight gain and increased mortality whereas dietary phytase increased feed intake and weight gain and decreased mortality. Supplemental phytase improved availabilities of dry matter, crude fat, ash, P, Zn, Mg, and Cu whereas dietary NPP level did not affect availabilities of nutrients except decreased Zn availability and increased Cu availability in reduced NPP diets. Nutrient retention of N, ash, Ca, P, Mg, and Zn were linearly decreased as dietary NPP levels reduced but dietary phytase increased their retention. Reduced dietary NPP increased ash excretion but decreased P and Cu excretion while dietary phytase decreased N excretion. Weight, length, girth and contents of ash, Ca, P and Mg of tibia linearly decreased as dietary NPP levels reduced. Dietary phytase increased length and ash content of tibia. It is concluded that dietary phytase can reduce P excretion and alleviate adverse affects caused by feeding low dietary NPP. Effects of phytase were greater in the lower NPP diets. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 2 : 250-257)*

Key Words : Broiler, Microbial Phytase, Non-Phytate Phosphorus, Growth Performance, Nutrients Availability

INTRODUCTION

Phytic acid has been regarded as the primary storage form of both phosphate and inositol in almost all seeds (Cosgraove, 1966). Approximately two third of P in the plant origin feedstuffs are in the form of phytate (Nelson et al., 1968). But the proportion of phytate P varies widely from 11.9% for tapioca to 83.1% for wheat bran (Paik, 2000). The P availability of phytate P is low, which lead to the use of inorganic P source to meet the P requirement of most monogastric animals such as poultry and pigs (Peeler, 1972). A negative influence of the phytic acid on the solubility of proteins and the function of pepsins can be also expected because of the ionic binding between the basic phosphate groups of phytic acid and protonised amino acid such as lysyl, histidyl and arginyl residues (Camus and Laporte, 1976; Singh and Krikorian, 1982). Phytate also complexes with proteins, making then less soluble (Smith and Rackis, 1957). Therefore, phytate-protein complexes are less subject to proteolytic digestion than the same protein alone (Hill and Tyler, 1954). When phytic acid is hydrolyzed by microbial phytase, it may release phytate-bound minerals (Sebastian et al., 1996b). The apparent N absorption of pig (Kornegay and Qian, 1994) and N retention of broilers (Yi et al., 1994) were improved when phytase was added to the diet. It has been reported that supplementary microbial phytase improves bioavailabilities of dietary P (Denbow et al., 1995; Simons et al., 1990; Ravindran et al., 1995; Um et al., 2000) and other minerals such as Ca, Mg, Cu, Zn, Fe and K, bound to phytate. (Eardaman, 1979; Um et al., 1999; Um and Paik, 1999). Phytase supplementation increases feed intake, body weight, and feed efficiency of broilers (Broz et al., 1994; Schoner et al., 1993; Sebastian et al., 1996a) and increases ash percentage in tibia (Broz et al., 1994; Nelson, 1971; Perney et al., 1993). Qian et al. (1996) and Um et al. (2000) reported phytase efficiency seemed more effective with a lower than higher NPP level in turkeys and broilers respectively.

The present experiment was conducted to determine the effects of phytase supplementation to the broiler diets with different levels of nonphytate phosphorus (NPP).

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** This research was supported by Dong Bang Co. Ltd for research fund and Chung-Ang University Special Research Grant (1999) for the purchase of Freeze Dryer.
Received August 23, 2000; Accepted September 28, 2000

MATERIALS AND METHODS

Animal and diet

Nine hundred sexed day old broiler chickens of Arbor Acres strain were randomly assigned to 18 floor pens. Each pen housed 50 birds of equal number in each sex. Three pens were assigned to each treatment.

Birds were given a starter diet from 0 to 3 weeks of age and a grower diet from 4 to 5 weeks of age. Formula and composition of experimental diets are shown in table 1. Diets were formulated to meet NRC (1994) requirements, except nonphytate P levels which differ among treatments. A 3×2 factorial arrangement of treatments was employed. There were three dietary NPP levels of control (C) (0.45% for starter diet and 0.35% for grower diet), C-0.1% NPP (0.35% for starter diet and 0.25% for grower diet), and C-0.2% NPP (0.25% for starter diet and 0.15% for grower diet) and two phytase levels (0 and 500 U/kg of diet). The source of phytase was a microbial phytase product of NOVO Nordisk Corp. Diets and water were fed *ad libitum*. Body weight and feed intake were recorded weekly on a pen base, and mortality was recorded.

Metabolic trial

At 4 wk of the feeding experiment, male birds kept for metabolic trial were individually housed in 24 metabolic cages. Four birds per treatment were given each experimental diet. After four days of an adjustment period, excreta were collected for 3 days. Feeds and excreta were analyzed for proximate components by AOAC (1990) methods. The dry-ashed samples were assayed for minerals by using ICP (inductively coupled plasma) Emission Spectrometer (Jobin Yvon, France). The availability of nutrients was calculated by dividing the amount of retained nutrient (ingested nutrient - excreted nutrient) with the amount of ingested nutrient.

Tibia parameters

On the completion of the feeding trial, 10 birds (5

Table 1. Formula and composition of experimental diets

	Starter (0-3 wks)						Grower (4-5 wks)					
	0.45% NPP[1]		0.35% NPP		0.25% NPP		0.35% NPP[1]		0.25% NPP		0.15% NPP	
	Phytase						Phytase					
Ingredients	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
	%											
Corn	58.37	58.37	58.37	58.37	58.37	58.37	65.28	65.28	65.28	65.28	65.28	65.28
Soybean meal (44% CP)	21.58	21.58	21.58	21.58	21.58	21.58	20.38	20.38	20.38	20.38	20.38	20.38
Corn Gluten	12.49	12.49	12.49	12.49	12.49	12.49	7.52	7.52	7.52	7.52	7.52	7.52
Rapeseed meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Tricalcium phosphate (18% P)	1.79	1.79	1.23	1.23	0.68	0.68	1.26	1.26	0.70	0.70	0.15	0.15
Limestone	0.76	0.76	1.24	1.24	1.72	1.72	0.97	0.97	1.45	1.45	1.93	1.93
Animal fat	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Broiler premix[2]	0.73	0.73	0.73	0.73	0.73	0.73	0.58	0.58	0.58	0.58	0.58	0.58
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.27	0.27	0.27	0.27	0.40	0.40
Methionine	0.14	0.14	0.14	0.14	0.14	0.14	0.03	0.03	0.03	0.03	0.03	0.03
Lysine-HCl	0.25	0.25	0.25	0.25	0.25	0.25	0.21	0.21	0.21	0.21	0.21	0.21
Sand	-	-	0.08	0.08	0.15	0.15	-	-	0.08	0.08	0.15	0.15
Phytase[3] (500 U/kg)	-	+	-	+	-	+	-	+	-	+	-	+
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calaulated composition												
ME, kcal/kg	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100
ME, MJ/kg	12.97	12.97	12.97	12.97	12.97	12.97	12.97	12.97	12.97	12.97	12.97	12.97
CP, %	23.0	23.0	23.0	23.0	23.0	23.0	20.0	20.0	20.0	20.0	20.0	20.0
Lysine, %	1.10	1.10	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00	1.00	1.00
Met+Cys, %	0.90	0.90	0.90	0.90	0.90	0.90	0.72	0.72	0.72	0.72	0.72	0.72
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90	0.90	0.90
Nonphytate P, %	0.45	0.45	0.35	0.35	0.25	0.25	0.35	0.35	0.25	0.25	0.15	0.15
Total P, %	0.72	0.72	0.62	0.62	0.52	0.52	0.61	0.61	0.51	0.51	0.41	0.41

[1] Conntrol: NPP level of control as 0.45% for 0-3 weeks and 0.35% for 4-5 weeks.

[2] Provides per kg of diet : vitamin A, 16,000 IU; vitamin D_3 3,200 IU; vitamin E, 0.4 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; niacin, 52 mg; folic acid, 1.3 mg; pantothenic acid, 19.5 mg; tocopherol, 30 IU; I, 0.5 mg; Zn, 50 mg; Mn, 70 mg; Fe, 80 mg; Cu, 10 mg; Se, 0.4 mg; Co, 0.4 mg.

[3] Provided by NOVO Nordisk Corp.

birds of median weight bird from each sex) per treatment were killed by cervical dislocation and left tibias were removed from birds. Flesh and soft tissues attached to the tibia were removed. After measuring the weight, length and girth, tibia were extracted with ether, ground and extracted again to the remove fat. The dry-ashed tibia were analyzed for minerals, as above.

Statistical analysis

Analyses of variance were made using General Linear Models procedure (SAS Institute, 1990) to determine the main effect and interaction of dietary NPP level and phytase. Significant differences among treatment means were tested by Duncan's multiple range test.

RESULTS

Growth performance is presented in table 2. Birds fed 0.2% less dietary NPP levels ate and gained less compared to birds of control and 0.1% less dietary NPP. Birds fed 0.1% less dietary NPP gained less in the starter period (0-3 wk) and overall (0-5wk) than control but there was no difference in feed intake. Reduced dietary NPP levels without phytase supplementation increased mortality, which was higher in the 0.2% less dietary NPP treatment than in the 0.1% less treatment. Phytase supplementation increased feed intake and weight gain and decreased mortality. Dietary NPP by phytase interactions were significant for feed intake and weight gain in the starter period (0-3 wk), mortality in the grower period (4-5 wk), and feed intake and mortality overall (0-5 wk). Low dietary NPP decreased Zn availability but increased Cu availability, whereas dietary phytase increased availabilities of DM, fat, fiber, P, Mg, Zn, and Cu (table 3), having a significant interaction with dietary NPP level. Lowering dietary NPP levels up to 0.2%, compared to control, linearly decreased retention of N, ash, Ca, P, Mg, Zn, and Cu and excretion of P and Cu but increased ash excretion (tables 4 and 5). Phytase supplementation increased retention of nutrients measured, and decreased N excretion. The NPP by phytase interactions were observed in retention of all nutrients measured and in excretion of N. Excretion of P decreased ($p<0.03$) as the level of NPP reduced. Phytase supplementation tended to decrease ($p<0.1$) P

Table 2. Body weight gain, feed intake, feed/gain and mortality of broiler chickens fed experimental diets

NPP[1]	Phytase	0 to 3 wks Feed intake	Weight gain	Feed/ gain	Morta -lity	4 to 5 wks Feed intake	Weight gain	Feed/ gain	Morta -lity	0 to 5 wks Feed intake	Weight gain	Feed/ gain	Morta -lity
		------- (g) ------			%	------- (g) -------			%	------- (g) -------			%
Control	0	842.7^{A}	586.9^{A}	1.44	5.39^{B}	1348.8^{A}	779.8^{A}	1.73^{b}	0.74^{B}	2191.5^{A}	1366.6^{A}	1.61	6.07^{C}
	500 U	585.6^{A}	597.2^{A}	1.44	2.00^{B}	1332.4^{A}	767.5^{A}	1.74^{b}	1.65^{B}	2191.0^{A}	1364.6^{A}	1.61	3.07^{C}
C-0.1%	0	783.4^{AB}	531.0^{B}	1.48	10.06^{B}	1180.6^{A}	662.1^{A}	1.78^{ab}	5.23^{B}	1964.0^{A}	1193.1^{B}	1.65	14.83^{BC}
	500 U	812.7^{A}	558.8^{AB}	1.45	4.04^{B}	1340.6^{A}	766.3^{A}	1.75^{ab}	5.62^{B}	2153.2^{A}	1325.1^{AB}	1.62	9.42^{BC}
C-0.2%	0	585.0^{C}	370.5^{D}	1.58	25.88^{A}	687.9^{B}	413.0^{B}	1.68^{b}	35.58^{A}	1272.9^{C}	783.4^{C}	1.63	52.38^{A}
	500U	705.4^{B}	455.6^{C}	1.55	13.63^{B}	837.7^{B}	433.6^{B}	1.94^{a}	7.15^{B}	1543.1^{B}	889.1^{C}	1.74	19.75^{B}
	SEM	12.74	6.81	0.03	1.68	127.60	21.18	0.04	1.45	34.85	24.68	0.02	1.78
Main effect means													
NPP													
Control		850.6	592.0	1.44	3.69	1342.6	773.6	1.73	0.90	2191.2	1365.6	1.61	4.57
C-0.1% NPP		798.0	544.9	1.47	7.05	1260.6	714.2	1.77	5.42	2058.6	1259.1	1.64	12.13
C-0.2% NPP		645.2	413.0	1.57	19.76	762.8	423.3	1.81	21.37	1408.0	836.3	1.69	36.07
Phytase													
0 U/kg		737.0	496.1	1.50	13.78	1072.4	618.3	1.73	13.85	1809.5	114.4	1.63	24.43
500 U/kg		792.2	537.2	1.49	6.55	1170.2	655.8	1.82	4.61	1962.4	1192.9	1.66	10.75
Analysis of variance		----------	----------	----------	----------	----------	(Probabilities)	----------	----------	----------	----------	----------	----------
NPP		0.0001	0.0001	0.0288	0.0001	0.0001	0.0001	0.4255	0.0001	0.0001	0.0001	0.1373	0.0001
Phytase		0.0042	0.0004	0.7074	0.0043	0.0132	0.1683	0.1354	0.0002	0.0038	0.0226	0.3261	0.0001
NPP×Phytase		0.0332	0.0081	0.9471	0.2263	0.1068	0.2016	0.0521	0.0001	0.0686	0.2131	0.1490	0.0002

[1] % of dietary NPP: Control; 0.45% NPP for starter and 0.35% NPP for grower, C-0.1%; 0.35% for starter and 0.25% for grower, C-0.2%; 0.25% for starter and 0.15% for grower.

$^{A\text{-}B,\ a\text{-}b}$ Means within each column with no common superscript differ significantly ($^{A\text{-}B}p<0.01$, $^{a\text{-}b}p<0.05$).

Table 3. Nutrient availability

NPP[1]	Phytase	DM	Protein	Fat	Ash	Fiber	NFE	Ca	P	Mg	Zn	Cu
		%										
Control	0	83.4^{A}	69.6^{A}	83.7^{C}	39.5^{ab}	34.4^{ab}	84.0	42.8^{b}	50.5^{ab}	19.8^{c}	27.0^{bc}	14.4^{B}
	500 U	84.7^{A}	73.4^{A}	89.1^{A}	47.7^{a}	37.0^{ab}	83.2	57.6^{a}	61.6^{a}	31.3^{a}	43.7^{a}	34.5^{A}
C-0.1%	0	83.8^{A}	66.2^{A}	85.6^{BC}	39.4^{ab}	36.2^{ab}	84.6	47.6^{ab}	57.3^{ab}	21.8^{bc}	31.1^{ab}	29.8^{AB}
	500 U	84.2^{A}	68.7^{A}	87.7^{AB}	45.1^{a}	42.1^{a}	85.2	44.8^{ab}	66.1^{a}	24.8^{abc}	30.2^{ab}	38.3^{A}
C-0.2%	0	79.7^{B}	57.6^{B}	85.0^{BC}	28.5^{b}	27.6^{b}	84.2	42.2^{b}	38.9^{b}	21.5^{bc}	13.3^{c}	26.1^{AB}
	500 U	84.7^{A}	68.3^{A}	84.8^{BC}	37.3^{ab}	45.9^{a}	83.6	46.8^{ab}	60.1^{a}	28.8^{ab}	24.7^{bc}	38.4^{A}
	SEM	0.65	2.58	0.65	3.49	3.00	0.67	3.07	4.60	2.08	3.68	2.84
Main effect means												
NPP												
Control		83.9	70.8	86.4	42.8	35.7	83.6	50.2	56.1	25.5	35.4	24.4
C-0.1% NPP		84.1	67.4	86.6	42.3	39.1	84.9	46.2	61.7	23.3	30.6	34.0
C-0.2% NPP		82.2	63.0	84.9	32.9	38.1	83.8	44.5	49.4	25.1	25.6	32.3
Phytase												
0 U/kg		82.4	64.4	84.8	35.8	33.5	84.3	44.2	48.8	21.0	28.2	23.4
500 U/kg		84.5	69.7	87.2	42.8	41.6	83.9	49.7	62.6	28.3	32.9	37.1
Analysis of variance		(Probabilities)										
NPP		0.076	0.089	0.110	0.081	0.618	0.317	0.343	0.189	0.683	0.001	0.033
Phytase		0.007	0.076	0.002	0.078	0.014	0.726	0.108	0.016	0.004	0.013	0.001
NPP × Phytase		0.031	0.410	0.011	0.940	0.129	0.690	0.109	0.592	0.307	0.001	0.283

[1] % of dietary NPP: Control; 0.45% NPP for starter and 0.35% NPP for grower, C-0.1%; 0.35% for starter and 0.25% for grower, C-0.2%; 0.25% for starter and 0.15% for grower.

A-B, a-b Means within each column with no common superscript differ significantly ($^{A-B}p<0.01$, $^{a-b}p<0.05$).

Table 4. Nutrient retention

NPP[1]	Phytase	Nitrogen	Ash	Ca	P	Mg	Zn	Cu
		Retention						
		g/bird/d					mg/bird/d	
Control	0	3.21^{AB}	2.54^{B}	0.47^{B}	0.35^{B}	0.035^{B}	3.52^{B}	0.25^{B}
	500 U	3.32^{A}	3.49^{A}	0.95^{A}	0.56^{A}	0.063^{A}	7.82^{A}	0.83^{A}
C-0.1%	0	2.60^{B}	2.40^{B}	0.61^{B}	0.33^{B}	0.036^{A}	4.00^{B}	0.65^{A}
	500 U	2.41^{B}	2.41^{B}	0.54^{B}	0.30^{BC}	0.041^{B}	3.53^{B}	0.82^{A}
C-0.2%	0	0.92^{C}	0.76^{C}	0.23^{C}	0.12^{D}	0.015^{C}	0.77^{C}	0.20^{B}
	500 U	2.75^{AB}	2.01^{B}	0.49^{B}	0.25^{C}	0.053^{AB}	2.58^{B}	0.79^{A}
	SEM	0.117	0.152	0.031	0.013	0.004	0.322	0.058
Main effect means								
NPP								
Control		3.27	3.01	0.71	0.45	0.049	5.67	0.54
C-0.1% NPP		2.68	2.45	0.58	0.32	0.039	3.77	0.73
C-0.2% NPP		1.97	1.38	0.36	0.18	0.034	1.67	0.49
Phytase								
0 U/kg		2.36	1.90	0.43	0.25	0.029	2.76	0.36
500 U/kg		2.97	2.67	0.66	0.37	0.052	4.64	0.81
Analysis of variance		(Probabilities)						
NPP		0.0001	0.0001	0.0001	0.0001	0.0112	0.0001	0.0130
Phytase		0.0001	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001
NPP × Phytase		0.0001	0.0029	0.0001	0.0001	0.0079	0.0001	0.0201

[1] % of dietary NPP: Control; 0.45% NPP for starter and 0.35% NPP for grower, C-0.1%; 0.35% for starter and 0.25% for grower, C-0.2%; 0.25% for starter and 0.15% for grower.

A-B, a-b Means within each column with no common superscript differ significantly ($^{A-B}p<0.01$, $^{a-b}p<0.05$).

Table 5. Nutrient excretion

		Excretion						
NPP[1]	Phytase	Nitrogen	Ash	Ca	P	Mg	Zn	Cu
		g/bird/d					mg/bird/d	
Control	0	1.13^{b}	3.12^{b}	0.50^{b}	0.27^{A}	0.12	7.63^{b}	1.18^{ABC}
	500 U	0.99^{b}	3.15^{b}	0.57^{ab}	0.29^{A}	0.11	8.30^{ab}	1.31^{AB}
C-0.1%	0	1.23^{ab}	3.40^{ab}	0.62^{ab}	0.23^{AB}	0.12	8.14^{ab}	1.40^{A}
	500 U	0.98^{b}	3.43^{ab}	0.62^{ba}	0.19^{AB}	0.11	7.52^{b}	1.21^{ABC}
C-0.2%	0	1.50^{a}	3.98^{a}	0.68^{a}	0.22^{AB}	0.13	9.29^{a}	1.10^{BC}
	500 U	1.19^{ab}	3.44^{ab}	0.57^{ab}	0.15^{B}	0.12	7.04^{b}	0.99^{C}
	SEM	0.043	0.181	0.035	0.019	0.006	0.401	0.059
Main effect means								
NPP								
Control		1.14	3.13	0.54	0.28	0.114	7.69	1.241
C-0.1% NPP		1.12	3.41	0.62	0.21	0.115	7.83	1.306
C-0.2% NPP		1.32	3.67	0.62	0.19	0.123	8.17	1.044
Phytase								
0 U/kg		1.32	3.45	0.59	0.24	0.121	8.35	1.227
500 U/kg		1.06	3.33	0.58	0.21	0.114	7.62	1.167
Analysis of variance		(Probabilities)						
NPP		0.1800	0.0724	0.1083	0.0031	0.4885	0.8076	0.0025
Phytase		0.0166	0.4279	0.7013	0.1069	0.3016	0.0930	0.2623
NPP × Phytase		0.9705	0.3940	0.1396	0.2456	0.8124	0.0355	0.0456

[1] % of dietary NPP: Control; 0.45% NPP for starter and 0.35% NPP for grower, C-0.1%; 0.35% for starter and 0.25% for grower, C-0.2%; 0.25% for starter and 0.15% for grower.

A-B, a-b Means within each column with no common superscript differ significantly ($^{A-B}p<0.01$, $^{a-b}p<0.05$).

excretion. Weight, length and girth of tibia linearly decreased as the dietary NPP level reduced, while supplemental phytase increased tibia length (table 6). The contents of ash, Ca, P, and Mg of tibia decreased as the dietary NPP level reduced while tibia ash was increased by phytase supplementation.

DISCUSSION

Lowering NPP level in a broiler diet decreased feed intake and weight gain, and increased feed conversion ratio and mortality. Supplemental phytase to low NPP diets improved those parameters. Similar observations of improved body weight by phytase supplementation have been reported by Broz et al. (1994) and Simons et al. (1990).

During the starter period, 0.1 and 0.2% reductions in dietary NPP level resulted in depressions of feed intake by 7.03 and 30.58% and weight gain by 9.52 and 36.86%, respectively. Phytase supplementation to the reduced NPP diets improved feed intake by 3.7 and 20.6% and weight gain by 5.2 and 23%, respectively. These results show that phytase is more effective with a lower than higher NPP level. Qian et al. (1996) reported similar result in the study involving turkey. Wise (1983) suggested that extra NPP might inhibit mucosal phytase activity in the small intestine of chickens. The 0.2% less NPP diet without supplemental phytase showed highest mortality throughout the experimental period, and this was decreased by phytase supplementation. This result is in agreement with a report by Denbow et al. (1995). The improvements in growth performance observed in phytase treatments may be due to the release of minerals from the phytate bound mineral and the utilization of inositol, as suggested by Knuckles and Betschart (1987), or increased availabilities of nutrients. Namkung and Leeson (1999) reported that phytase supplementation to broiler diet containing low available phosphorus increased body weight, AME, and availability of total amino acids. Dietary phytase tended to increase protein availability although this was not different among treatments. Supplemental phytase increased N retention by 36.4% and decreased N excretion up to 19.7%. Increased N retention by phytase was also observed in previous studies with broilers (Yi et al., 1994; Sazzad et al., 1995) and laying hens (Van der Klis and Versteegh, 1995). Availabilities and retention of P, Mg, Zn and Cu were increased by phytase supplementation. Lie et al. (1993) and Yi et al. (1996) reported that supplemental microbial phytase improved the bioavailability and

Table 6. Weight, length, girth and mineral content for tibia

NPP[1]	Phytase	Weight	Length	Girth	Ash	Ca	P	Mg
		g	------------ cm	---------	%	---------- % of tibia ash		-----------
Control	0	9.41^{A}	9.04^{A}	2.58^{a}	52.1^{A}	17.27^{AB}	8.83^{AB}	0.56^{A}
	500 U	9.19^{A}	8.66^{AB}	2.63^{a}	55.1^{A}	18.45^{A}	9.37^{A}	0.58^{A}
C-0.1%	0	6.80^{B}	8.45^{B}	2.56^{a}	44.6^{B}	14.65^{C}	7.70^{C}	0.50^{BC}
	500 U	6.63^{B}	8.56^{B}	2.59^{a}	53.8^{A}	17.60^{AB}	9.03^{AB}	0.54^{AB}
C-0.2%	0	2.46^{D}	6.81^{D}	2.31^{b}	46.7^{B}	16.01^{BC}	8.12^{BC}	0.49^{C}
	500 U	3.67^{C}	7.58^{C}	2.51^{ab}	46.9^{B}	14.22^{C}	7.26^{C}	0.46^{C}
	SEM	0.29	0.13	0.09	1.24	0.66	0.31	0.01
Main effect means								
NPP								
Control		9.30	8.85	2.61	53.6	17.86	9.06	0.57
C-0.1% NPP		6.72	8.50	2.58	49.2	16.12	8.36	0.52
C-0.2% NPP		3.07	7.20	2.41	46.9	15.13	7.71	0.47
Phytase								
0 U/kg		6.22	8.10	2.48	47.8	16.00	8.19	0.52
500 U/kg		6.50	8.27	2.58	52.0	16.80	8.57	0.53
Analysis of variance		------------------------			(Probabilities)	------------------------		
NPP		0.0001	0.0001	0.0243	0.0001	0.0001	0.0001	0.0001
Phytase		0.1489	0.0496	0.1293	0.0001	0.0664	0.0605	0.3160
NPP × Phytase		0.0036	0.0001	0.4610	0.0001	0.0002	0.0002	0.0455

[1] % of dietary NPP: 0.45% NPP for starter and 0.35% NPP for control; 0.35% for starter and 0.25% for finisher for C-0.1% NPP; 0.25% for starter and 0.15% for finisher for C-0.2% NPP.

[A-B, a-b] Means within each column with no common superscript differ significantly ($^{A-B}p<0.01$, $^{a-b}p<0.05$).

retention of Zn in broiler and pigs. Sebastian et al. (1996a) reported that supplementation with phytase also significantly increased the relative retention of Cu. The increase of P availability in reduced NPP diets by phytase supplementation was higher in 0.2% less dietary NPP level than in control or 0.1% less dietary NPP level. Improvement of P availability by phytase supplementation was greater in lower NPP diets (Um et al., 2000). Sanders et al. (1992) reported that birds showed the highest rate of phytate hydrolysis when birds were fed diets containing low dietary level of NPP with phytase. Increased availability of P resulted in increased retention and reduced excretion of P. Reduction of P excretion was greater in lower NPP diets with phytase supplementation. Reduction of P excretion was 45% with the 0.2% less NPP diet with phytase supplementation, and 30% for 0.1% less NPP with phytase supplementation. Phytase supplementation increased tibia length and tibia ash, an observation that agrees with previous studies involving chickens (Nelson, 1971; Broz et al., 1994). Phytase supplementation to 0.1% less NPP diets increased the content of P, Ca and Mg in tibia but phytase did not affect them with the 0.2% less NPP diet. These results show that phytase supplementation of a diet too low in NPP may not overcome lowering of mineral content in tibia caused by low NPP. Broz et al. (1994) showed that phytase supplementation of a low phosphorus diet increased the tibia ash percentage in broiler chickens but there was no difference in P and Ca concentration. However, Beihl (1995) reported increased P content in tibia of broiler chicks fed diet containing a normal phosphorus level with phytase.

It is concluded that adverse affects of low NPP can be alleviated by supplementation with phytase, and that effects of phytase were greater in the lower NPP diet. Lowering 0.1% NPP in a broiler diet had no significant affects on performance, but reduced P excretion by 30% when microbial phytase was supplemented at the level of 500 U/kg diet.

REFERENCES

AOAC. 1990. Offical Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arligton, VA.

Biehl, R. R., D. H. Baker and H. F. Deluca. 1995. α-hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese untilization in chicks fed soybased diets. J. Nutr. 125:2407-2416.

Broz, J., P. Oldale, A. H. Perrin-Voltz, G. Rychen, J. Schulze and C. Simoes Nunes. 1994. Effect of supplemental phytase on performance and phosphorus utilization in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. Br. Poult. Sci. 35:273-280.

Camus, M. C. and J. C. Laporte. 1976. Inhibition de la proteolyse pepsique en vitro par de ble. Role de l'acide phytique des issues. Ann. Biol Biochem. Biophys 16:719-729.

Cosgrove, D. 1966. The chemistry and biochemistry of inositol polyphosphates. Rev. Pure Appl. Chem. 16:209-215.

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.

Eardaman, J. W. Jr. 1979. Oil seed phytates: nutritional implications. J. Intern. Med. 77:760-764.

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

Knuckles, B. E. and A. A. Betschart. 1987. Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. J. Food Sci. 52:719-721.

Kornegay, E. T. and H. Qian. 1994. Effectiveness of Natuphos phytase as influenced by dietay phosphorus for improving the availability of phytate phosphorus in a corn-soybean meal based diet fed to young pigs. J. Anim. Sci. 72(Suppl. 1):330(Abstr).

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. 3:1117-1123.

Namkung. H. and S. Leeson. 1999. Effect of phytase enzyme on dietary nitrogen corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler diets. Poult. Sci. 78:1317-1319.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th, rev. ed. National Academy Press, Washington, DC.

Nelson, T. S., T. R. Shieh, R. J. Wodzinski and J. H. Ware. 1968. The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. Poult. Sci. 47:1842-1848.

Nelson, T. S., T. R. Shieh, R. J. Wodzinski and J. H. Ware. 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. J. Nutr. 101:1289-1294.

Paik, I. K. 2000. Nutritional management for evironment friendly animal production. Asian-Aust. J. Anim. Sci. 13 (Special Issue):302-314.

Peeler, H. T. 1972. Biological availability of nutrients in feeds: availability of major mineral ions. J. Anim. Sci. 77:83-89.

Perney, K. M., A. H. Cantor, M. L. Straw and K. L. Herkelman. 1993. The effect of dietary phytase on growth performance and phosphorus utilization of broiler chickens. Poult. 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 phosphorus ratios and phosphorus levels. Poult. Sci. 75:69-81.

Ravindran, V., D. M. Denbow, E. T. Kornegay, Z. Yi and R. J. Hulet. 1995. Supplemental phytase improves availability of phosphorus in soybean meal for turkey poults. Poult. Sci. 74:1843-1854.

Sandberg, A. S., T. Larsen and B. Sandstrom. 1993. High dietary calcium levels decrease colonic phytate degradation in pig. J. Nutr. 123:559-566.

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. 1988. SAS User's Guide: Statistics. Version 6 Edition. SAS Institute Inc., Cary, NC.

Sazzad, M. H., P. E. N. Givisiez and C. G. Silva. 1995. Effect of dietary protein, phosphorus and phytase on performance and phosphorus utilization of broiler chicks. Poult. Sci. 74(Suppl. 1):109(Abstr.).

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.

Sebastian, S., S. P. Touchburn, E. R. Chavez 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. Poult. Sci. 75:729-736.

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

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.

Singh, M. and A. D. Krikorian. 1982. Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem. 30:799-800.

Smith, A. K. and J. J. Rackis. 1957. Phytin elimination in soybean protein J. Am. Chem. Soc. 79:633-637.

Um, J. S., H. S. Lim, S. H. Ahn and I. K. Paik. 2000. Effects of microbial phytase supplementation to low phosphorus diets on the performance and utilization of nutrients in broiler chickens. Asian-Aust. J. Anim. Sci. 13(6):824-829.

Um, J. S. and I. K. Paik. 1999. Effects of microbial phytase supplementation on egg production, eggshell quality, and mineral retention of laying hens fed different levels of phosphorus. Poult. Sci. 78:75-79.

Um, J. S., I. K. Paik, M. B. Chang and B. H. Lee. 1999. Effects of microbial phytase supplementation to diets with low non-phytate phosphorus levels on the performance and bioavailability of nutrients in laying hens. Asian-Aust. J. Anim. Sci. 12(2):203-208.

Van der Klis, J. D. and H. A. J. Versteegh. 1991. Ileal absorption of P in lightweight white laying hens using microbial phytase and various calcium contents in laying hen feed. Spelderholt Pub. No. 563. Het Spelderholt, Wageningen, The Netherlands.

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

Yi, Z., E. T. Kornegay, V. Ravindran and D. M. Denbow. 1994. Improving availability of corn and soybean meal P

for broilers using Natuphos phytase and calculation of replacement values of inorganic P by phytase. Poult. Sci. 73(Suppl. 1):89(Abstr.).
Yi, Z., E. T. Kornegay and D. M. Denbow. 1996. Improving phytate phosphorus availability in corn and soybean meal for broiler using microbial phytase and calculation of phosphorus equivalency values of phytase. Poult. Sci. 75:240-249.