Effects of Microbial Phytase Supplementation to Low Phosphorus Diets on the Performance and Utilization of Nutrients in Broiler Chickens[a]

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ABSTRACT : A five wk feeding experiment was conducted with day-old one thousand broiler chicks (Arbor Acres) to determine the effects of microbial phytase (Natuphos®) supplemented to low nonphytate P (NPP) corn-soy diets. Five pens of 50 mixed sex birds each were randomly assigned to each of the four dietary treatments: T1, control diet containing normal NPP level; T2, T1-0.1% NPP+600 U of phytase/kg diet; T3, T1 - 0.2% NPP+600 U of phytase/kg diet; and T4, T1-0.3% NPP+600 U of phytase/kg diet. T1, T2, and T3 showed similar growth rate, feed intake, and feed efficiency, indicating that NPP level in broiler diets could be reduced by approximately 0.2% by the microbial phytase supplementation. But T4 showed significantly ($p<0.05$) lower weight gain than others. The phytase supplementation improved P availability resulting in low P excretion. Weight and girth of metatarsal bone were increased by phytase supplementation at low NPP diet treatments but ash contents were not significantly different. It can be concluded that NPP level of corn-soy broiler diets can be safely lowered by approximately 0.2% by supplementing 600 U of microbial phytase/kg diet. With the adjusted level of NPP and phytase supplementation, P excretion could be reduced by 50%. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 6 : 824-829*)

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

INTRODUCTION

Phytate P is the major storage form of P in almost all seed plants. This phytate P has low P availability (National Research Council, 1994), which lead to the use of inorganic P sources to meet the P requirement of poultry (Yi et al., 1996a, b, c). Therefore, large amounts of P are unabsorbed and excreted through poultry excreta. Moreover, phytate P is known to lower the bioavailability of other essential minerals, because the phytic acid acts as the binder to build the mixed mineral salts with various divalent cations, such as Ca, Mg, Zn, Fe and K (Cosgrove, 1980; Reddy et al., 1982).

From the common corn-soy diets, in which no native phytase activity could be identified, only 29% of the total phosphorus consumed is known to be absorbed (Pallauf and Rimbach, 1992). In previous studies using corn-soy diets, several researchers have shown that dietary supplementation of broiler diets with microbial phytase improved the availability or retention of P and Ca (Swick and Ivey, 1990; Simons et al., 1992; Hoppe and Schwarz, 1993) and reduced N excretion (Yi et al., 1996c). Roberson and Edward (1984) also reported that supplementation of phytase increased the tibia Zn concentration but did not improve the apparent Zn retention in broiler chicks.

Several researchers observed that the utilization of phytate P from poultry feed was significantly increased when the phytase enzyme at a level of 250 to 1,050 U/kg of diet was incorporated into the feed (Peterson, 1993; Ravindran et al., 1993; Sebastian et al., 1996a, b; Yi et al., 1996a). Also, the maximum growth response of broilers to the phytase addition was reduced with increasing levels of dietary non-phytate phosphorus (NPP) (Kornegay et al., 1996). The objective of this experiment is to investigate the effects of microbial phytase supplementation to the diets containing gradually lower NPP levels by reducing tricalcium phosphate (TCP) on the growth performance and nutrient utilization and excretion of broiler chickens.

MATERIALS AND METHODS

Experimental diet

The formula and chemical compositions of broiler starter (1 to 21 d) and grower (22 to 35 d) diets are shown in table 1 and 2, respectively. Diets were formulated to be iso-caloric and iso-nitrogenous but levels of NPP and total P were different. Different levels of TCP were used in diets to adjust the P levels. Limestone and acid-washed sand were used to provide necessary Ca level and volume. The source of phytase was microbial phytase product (Natuphos®: provided by BASF Korea Ltd., Seoul, 100-611, Korea).

Experimental design and feeding trial

Day-old one thousand commercial broiler chicks

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(Arbor Acres®: provided by Hanil Poultry Farm Co., Ltd., Osan-Si, Kyonggi-Do, Korea) were randomly allotted to twenty floor pens in which 50 birds, 25 birds in each sex, per pen were housed. The experiment consisted of four dietary treatments with 5 replicates (pens) each. Control diet (T1) contained normal level of NPP (starter: 0.45%, grower: 0.40%). T2 diet contained approximately 0.1% less NPP (0.11% less in starter diet and 0.09% less in grower diet or one third less dietary TCP) than the control diet but 600 U of phytase/kg diet was supplemented. T3 diet contained approximately 0.2% less NPP (0.22% less in starter diet and 0.18% less in grower diet or two third less dietary TCP) but supplemented with 600 U of phytase/kg diet. T4 diet contained approximately 0.3% less NPP (0.33% less in starter diet and 0.27% less in grower diet or none dietary TCP) but supplemented with 600 U of phytase/kg diet. Diets were offered in mash form, and feed and water were given *ad libitum* during experimental periods. The house was lighted for 24 h a day. The feeding trial was carried out for 35 days, during which body weight and feed consumption were measured weekly.

Availability and excretion of nutrients

At the end of the feeding trial, 3 birds from each treatment were randomly assigned to individual metabolic cages to determine the availability of nutrients of experimental diets. Excreta from the birds were collected for 3 days. Foreign substances (feathers, scurf, etc) intermixed within the collected excreta were eliminated before drying at 60℃ for 48 hr and subsequent grinding. Proximate composition of feeds and feces were analyzed by the methods of AOAC (1990). The availability of nutrients was calculated by dividing the amount of retained nutrient (ingested nutrient - excreted nutrient) with the amount of ingested nutrient. To determine content of minerals, such as Ca, P, Mg, Zn, Fe, and Cu, samples were prepared by dry washing method (AOAC, 1990) and

Table 1. Formula and composition of broiler starter diets

Ingredients	Treatments			
	T1	T2	T3	T4
	(%)			
Corn	60.69	60.69	60.69	60.69
Soybean meal (44% CP)	26.30	26.30	26.30	26.30
Corn gluten meal	7.44	7.44	7.44	7.44
Tricalcium phosphate	1.80	1.20	0.60	-
Animal fat	1.50	1.50	1.50	1.50
Limestone	0.75	1.27	1.79	2.31
Broiler premix[1]	0.73	0.73	0.73	0.73
Salt	0.40	0.40	0.40	0.40
DL-Methionine (50%)	0.23	0.23	0.23	0.23
Lysine-HCl (78%)	0.17	0.17	0.17	0.17
Sand	-	-	0.07	0.18
Natuphos[2]	-	+	+	+
Total	100.00	100.00	100.00	100.00
Calculated composition				
ME, kcal/kg	3050	3050	3050	3050
Crude protein	21.5	21.5	21.5	21.5
Lysine	1.10	1.10	1.10	1.10
Methionine+cystine	0.86	0.86	0.86	0.86
Calcium	1.00	1.00	1.00	1.00
Non-phytate phosphorus	0.45	0.34	0.23	0.12
Total phosphorus	0.71	0.60	0.50	0.39

[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] A microbial phytase product (500 U/g) provided by BASF Korea Ltd. Supplemented groups contained 600 U microbial phytase/kg diet.

Table 2. Formula and composition of broiler grower diets

Ingredients	Treatments			
	T1	T2	T3	T4
	(%)			
Corn	68.53	68.53	68.53	68.53
Soybean meal (44% CP)	18.85	18.85	18.85	18.85
Corn gluten meal	7.59	7.59	7.59	7.59
Tricalcium phosphate	1.56	1.04	0.52	-
Animal fat	1.50	1.50	1.50	1.50
Limestone	0.75	1.20	1.65	2.10
Broiler premix[1]	0.58	0.58	0.58	0.58
Salt	0.29	0.29	0.29	0.29
DL-Methionine (50%)	0.28	0.28	0.28	0.28
Lysine-HCl (78%)	0.07	0.07	0.07	0.07
Sand	-	0.07	0.14	0.21
Natuphos[2]	-	+	+	+
Total	100.00	100.00	100.00	100.00
Calculated composition				
ME, kcal/kg	3150	3150	3150	3150
Crude protein	19.0	19.0	19.0	19.0
Lysine	1.00	1.00	1.00	1.00
Methionine+cystine	0.71	0.71	0.71	0.71
Calcium	0.90	0.90	0.90	0.90
Non-phytate P	0.40	0.31	0.22	0.13
Total P	0.64	0.55	0.45	0.36

[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; 0.5 mg; Zn, 50 mg; Mn, 70 mg; Fe, 80 mg; Cu, 10 mg; Se, 0.4 mg.

[2] A microbial phytase product (500 U/g) provided by BASF Korea Ltd. Supplemented groups contained 600 U microbial phytase/kg diet.

Table 3. Body weight gain, feed intake, feed/gain and mortality of broiler chickens fed experimental diets from 1 to 35 days

Item	age (day)	Treatments[1]				SEM
		T1	T2	T3	T4	
Weight gain, g/bird	1 - 21	718.5[b]	748.1[a]	723.9[b]	681.2[c]	5.17
	22 - 35	896.3	883.2	879.8	880.4	10.66
	1 - 35	1,614.8[a]	1,631.3[a]	1,603.6[a]	1,561.6[b]	12.76
Feed intake, g/bird	1 - 21	1,075.9[bc]	1,108.2[a]	1,097.2[ab]	1,052.3[c]	7.89
	22 - 35	1,751.4	1,751.1	1,740.5	1,718.9	16.81
	1 - 35	2,827.3[ab]	2,859.3[a]	2,837.7[ab]	2,771.2[b]	23.16
Feed/gain (g/g)	1 - 21	1.50[b]	1.48[b]	1.52[ab]	1.55[a]	0.01
	22 - 35	1.96	1.98	1.98	1.95	0.02
	1 - 35	1.75	1.75	1.77	1.77	0.01
Mortality, %	1 - 21	3.60	3.20	1.60	4.00	1.16
	22 - 35	1.26	0.82	1.22	2.47	0.74
	1 - 35	4.80	4.00	2.80	6.40	1.29

[1] T1=control diet containing normal nonphytate P (NPP) level, T2=control diet - 0.1% NPP+600 U of phytast/kg diet, T3=control diet - 0.2% NPP+600 U of phytase/kg diet, T4=control diet - 0.3% NPP+600 U of phytase/kg diet.
[a,b,c] Values with different superscripts in the same row are significantly different ($p<0.05$).

concentrations were measured at specific wavelength 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, Longumeau, Cedex, 91165, France).

Bone parameters

On the termination of the feeding trial, fifteen birds from each treatment were sacrificed by asphyxiation, and the left metatarsal bones were removed and dried at 60℃ for 72 h. The weight, length, and girth of the metatarsal bones were measured before grinding and fat extraction. The fat-free dry bones were ashed to determine crude ash content.

Statistical analysis

The data obtained from the experiment were analyzed by using the General Linear Models (GLM) of SAS (SAS Institute, 1985). Significant differences between means of each treatment were determined at $p<0.05$ using Duncan's new multiple range test (Duncan, 1955). Least Square Means (LSM) was also used to include the final body weight as a covariate in analyses of weight, length, and girth of bones.

RESULTS AND DISCUSSION

The results of feeding trial are shown in table 3. Weight gain, feed intake and feed/gain were significantly ($p<0.05$) influenced by treatment during starter period (1 to 21 d). Weight gain during the overall periods (1 to 35 d) showed significant differences among the treatments. T4 showed the lowest ($p<0.05$) weight gain among treatments while T1, T2, and T3 were not significantly different.

The data of feed intake showed similar trend to that of body weight gain. Feed to gain ratio of T4 was significantly ($p<0.05$) higher than those of T1 and T2 during 1 to 21 days, but was not significantly different during the grower (22 to 35 d) or overall period. No significant differences were found in mortality among treatments. The results showed that BW gain of broilers fed diet low in NPP (0.34%) with 600 U of microbial phytase/kg diet (T2) was significantly greater than that of T1 (control) which contains NRC (1994) recommendation (0.45% NPP) for the first 21 days. Lowering NPP level to 0.23% or reducing dietary TCP by two third with phytase supplementation (T3) did not affect BW gain compared to the control (T1) during starter period. But complete removal of TCP or lowering NPP level to 0.12% with phytase (T4) significantly reduced BW gain during starter period. The BW gains from 22 to 35 d of age (grower period) were not different among treatments, however. This result may indicate that the phytase supplementation is more effective or growth response to treatments is more sensitive to NPP level in early growing stage of broilers. Sebastian et al. (1996b) demonstrated that the inability of young broiler chickens to utilize phytic acid leads to slow growth rate and lower concentration of plasma phosphorus. Kornegay et al. (1996) reported that the maximum growth responses were observed by the addition of 600 U of phytase/kg diet in broiler diet containing 0.34% NPP. These findings are in agreement with our results that T2 (0.34% NPP plus 600 U of phytase/kg diet) showed the best performances on BW gain, feed intake and feed/gain

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

Nutrients	Treatments[1]				SEM
	T1	T2	T3	T4	
	(%)				
DM	80.1	82.0	81.7	80.9	0.79
Crude protein	72.4	72.9	73.0	72.3	1.81
Ether extract	80.2	84.3	84.3	83.1	1.44
Crude fiber	14.6[ab]	20.5[a]	13.0[b]	11.8[b]	2.01
Crude ash	35.9	38.3	34.8	37.6	1.71
NEF	90.4	92.0	92.2	91.2	0.60
Calcium	30.1	27.8	31.9	29.5	3.60
Phosphorus	29.4[b]	35.6[ab]	40.5[a]	43.7[a]	2.53
Magnesium	14.2	19.3	20.4	15.1	2.30
Zinc	24.0	26.8	26.4	31.0	2.44
Iron	4.3[b]	14.2[a]	25.7[a]	9.0[ab]	2.60
Copper	5.9	24.9	13.5	6.4	4.39

[1] T1=control diet containing normal nonphytate P (NPP) level, T2=control diet - 0.1% NPP+600 U of phytase/kg diet, T3=control diet - 0.2% NPP+600 U of phytase/kg diet, T4=control diet - 0.3% NPP+600 U of phytase/kg diet.
[a,b] Values with different superscripts in the same row are significantly different at p<0.05.

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

Nutrients	Treatments[1]				SEM
	T1	T2	T3	T4	
	(g/bird/d)				
Nitrogen	1.80	1.76	1.61	1.86	0.12
Ash	7.62	7.11	6.98	8.00	0.43
Calcium	1.21	1.26	1.06	1.28	0.11
Phosphorus	0.84[a]	0.65[b]	0.42[c]	0.36[c]	0.04
Magnesium	0.30	0.28	0.26	0.32	0.02
	(mg/bird/d)				
Zinc	15.73	14.39	13.09	16.15	0.95
Iron	36.39	32.28	29.47	36.24	2.14
Copper	10.88	10.52	9.52	10.95	0.58

[1] T1=control diet containing normal nonphytate P (NPP) level, T2=control diet - 0.1% NPP+600 U of phytase/kg diet T3=control diet - 0.2% NPP+600 U of phytase/kg diet, T4=control diet - 0.3% NPP+600 U of phytase/kg diet.
[a,b,c] Values with different superscripts in the same row are significantly different (p<0.05).

in starter period. It seems that the improved BW gain by the phytase supplementation was effected by increased feed intake. Denbow et al. (1996) also observed improvements in feed efficiency when phytase was added to the low P diets. Adding the graded levels of phytase to the basal diet linearly improved feed efficiency of broilers (Yi et al., 1996c). Simons et al. (1990) obtained a significant improvement in chick growth rate when supplementing a low-P diet with phytase at the levels between 375 and 2,000 U/kg of diet. They also reported an improvement in P Utilization and feed efficiency when broilers were fed 1,000 and 1,500 U of phytase/kg of diet. Results of the overall period indicated that BW gain and feed intake were not adversely affected by feeding 0.22-0.23% NPP diet or by removing two third of TCP in corn-soy diet if 600 U phytase/kg diet is supplemented. However, feeding 0.12-0.13% NPP diet or complete removal of TCP reduced BW gain and feed intake even with phytase supplementation. The results of availabilities of nutrients are shown in table 4. Among the proximate components, the availability of crude fiber in T2 was significantly (p<0.05) higher than those of T3 and T4, but not significantly different from T1. Availabilities of Ca, Mg, Zn, and Cu were not significantly different among treatments. The availability of P in phytase supplemented treatments (T2, T3 and T4) was

Table 6. Weight, length, girth and ash content of metatarsal bone of broiler chickens fed experimental diets for 35 days

Items	Treatments[1]				SEM
	T1	T2	T3	T4	
Weight[2], g in DM	3.15[b]	3.18[b]	3.46[a]	3.28[ab]	0.086
Length[2], cm	7.65	7.57	7.73	7.65	0.073
Girth[2], cm	2.44[b]	2.49[b]	2.73[a]	2.71[a]	0.076
Ash[2], %, fat-free DM	45.14	43.79	45.05	44.61	0.45

[1] T1=control diet containing normal nonphytate P (NPP) level, T2=control diet - 0.1% NPP+600 U/kg phytase, T3=control diet - 0.2% NPP+600 U of phytase/kg diet, T4=control diet - 0.3% NPP+600 U of phytase/kg diet.

[2] Least square means calculated with final body weight as the covariate.

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

significantly ($p<0.05$) greater than the control. Within the phytase supplemented groups, availability of P increased as the NPP level in the diet decreased. The Fe availability was significantly ($p<0.05$) increased in low P diets with phytase supplementation. Improved utilization of N and DM by supplementation of microbial phytase in broiler chickens was reported by Yi et al. (1996c). In the present study, however, improved availabilities of these nutrients were not significant (table 4). Schoner et al. (1991, 1993) reported improved Ca retention of broilers given supplemental phytase. This study showed that the availability of Ca was not affected by microbial phytase.

As shown in table 5, P excretion decreased significantly as the level of NPP decreased along with phytase supplementation. Excretions of N, ash, Ca, Mg, Fe, Zn, and Cu, however, were not different among treatments. Compared to T1, P excretion was reduced by 23, 50, and 57% at T2, T3 and T4, respectively. Such reductions may be the result of reduced dietary NPP level per se and better efficiency of phytase when the P level is low. These results are similar to the observations of Kornegay et al. (1996). They claimed that the supplementation of 200 to1,200 U of phytase/kg diet, at the range of 2.0 to 3.4 g NPP/kg diet, reduced P excretion by 25 to 54%. At the level of 700 U of phytase/kg diet with 0.27% NPP diet, the P excretion was reduced by 37% when compared with the 0.45% NPP diet (Yi et al., 1996a).

In table 6 are shown the data on weight, length, girth and ash content of left metatarsal bones. The metatarsal bone in T3 was heavier than that of T1 and T2. And the size of girth was also greater in T3 and T4 than in T1 and T2. Nelson et al. (1971) observed the improvement of bone ash in chicks fed diets with phytase supplementation. The increase in the bone ash content suggests the improvement in bone mineralization due to increased P or Ca utilization, which was caused by the liberation of inorganic P and Ca from the phytate molecule by the phytase enzyme in the digestive tract (Perney et al., 1993). However, the results from this experiment did not show significant difference in bone ash. Also, we do not have reasonable explanation why weight and girth of metatarsus tended to be greater in phytase supplemented low NPP treatments.

In conclusion, NPP level of broiler diet can be safely lowered by approximately 0.2% or two-third of dietary TCP can be saved by supplementing 600 U of microbial phytase/kg diet. At this level of NPP and supplementary phytase, P excretion could be reduced by 50% (0.42 g/bird/d).

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