Effects of Microbial Phytase Supplementation on Egg Production, Eggshell Quality, and Mineral Retention of Laying Hens Fed Different Levels of Phosphorus

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ABSTRACT A 20-wk feeding trial (21 to 40 wk of age) was conducted to evaluate the effects of phytase supplementation on egg production, egg quality, nutrient retention, and P excretion of laying hens fed diets containing different levels of P. Nine hundred and sixty ISA Brown® hens were randomly allocated to completely randomized block arrangement of four diets: corn-soybean diet (1.4% tricalcium phosphate, TCP) without (T1, control) and with phytase (T2); 0.7% TCP (T3) or 0% TCP (T4) diet with phytase. Dietary microbial phytase was added at a level of 500 U/kg. Both hen-day and hen-housed egg production of T2 were significantly ($P < 0.05$) higher than other treatments, which were not different among themselves. Egg weights were also significantly ($P < 0.05$) different among treatments ,with T2 being the highest. Feed consumption of T2 was significantly ($P < 0.05$) higher than other treatments but feed conversion ratio was not significantly different from others. Specific gravity and shell thickness of the eggs were highest in the control (T1) but eggshell strength and broken egg to total egg ratio were not different among treatments. Haugh units were not different among treatments. Retention of Ca, P, Mg, Fe, and Zn were greater ($P < 0.05$) in phytase-supplemented groups. There were significant ($P < 0.05$) differences in excretion of ash, P, and Zn. The excretion of these components were highest in the control, whereas P excretion was significantly lower in the T3 and T4 groups. In conclusion, supplementation of the microbial phytase to normal corn-soybean diet improved egg production and can reduce TCP level in the diet without affecting egg production and egg quality. Significant reduction of P excretion can be also achieved.

(*Key words*: phytase, phosphorus excretion, layer, egg production, egg quality)

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INTRODUCTION

Generally, corn and soybean meal are the major feedstuffs in the diets of poultry. Most plant seeds, including corn and soybeans, contain more than 60% of P in the form of phytate (Nelson *et al*., 1968; Reddy *et al*., 1982). This phytate P has low availability (NRC, 1994), which leads to the use of inorganic P sources to meet the P requirement of most monogastric animals such as poultry and pigs. A negative influence of 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 protonized amino acid such as lysyl, histidyl, and arginyl residues (De Rham and Jost, 1979; Fretzdorff *et al*., 1995).

Supplementation of high levels of phytase resulted in a lower equivalence per unit of phytase due to the significant quadratic phytase response in P absorption (Van der Klis and Versteegh, 1996). Van der Klis *et al*. (1994) reported that supplementation of 250 U of phytase/kg diet in laying hens was equivalent to 0.8 g of P from monocalcium phosphate (MCP). In a study with laying hens fed a corn-soybean basal diet, 500 U phytase/kg diet was shown to have an effect equivalent to 1 g of P (Peter and Jeroch, 1993). Gordon and Roland (1997) reported that hens consuming the low nonphytate P (NPP) diet with supplementary phytase performed as well as the hens fed diets containing higher levels of NPP without supplementary phytase.

This experiment was conducted to determine the effects of a microbial phytase supplementation on egg production, eggshell quality, and excretion of P and other nutrients in laying hens fed different levels of dietary P.

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Abbreviation Key: MCP = monocalcium phosphate; NPP = nonphytate phosphorus; TCP = tricalcium phosphate.

TABLE 1. Formula and composition of experimental diets

Ingredients[1]	Treatments[2] 1	2	3	4
	(%)			
Corn	62.20	62.20	62.20	62.20
Soybean meal (44% CP)	21.00	21.00	21.00	21.00
Limestone	8.55	8.55	9.16	9.76
Rapeseed meal	3.00	3.00	3.00	3.00
Animal fat	1.50	1.50	1.50	1.50
Wheat bran	1.53	1.43	1.43	1.43
Tricalcium phosphate (32% Ca, 18% P)	1.40	1.40	0.70	. . .
Layer premix[3]	0.32	0.32	0.33	0.32
Sodium chloride	0.31	0.31	0.31	0.31
DL-methionine (50%)	0.19	0.19	0.19	0.19
Sand	. . .	. . .	0.08	0.19
Natuphos[4] (500 U/g)	. . .	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated values				
ME, kcal/kg	2,750	2,750	2,750	2,750
CP	16.0	16.0	16.0	16.0
Calcium	3.75	3.75	3.75	3.75
Available phosphorus	0.37	0.37	0.24	0.12
Total phosphorus	0.61	0.61	0.49	0.37
Analyzed value[5]				
Dry matter	88.55	88.93	88.41	88.53
CP	17.83	17.93	17.87	17.97
Calcium	4.25	4.20	4.25	4.21
Total phosphorus	0.70	0.69	0.54	0.42

[1]As-fed basis.

[2]T1 = control diet (1.4% TCP), T2 = control diet + 500 U phytase/kg, T3 = 0.7% TCP diet + 500 U phytase/kg, T4 = 0% TCP diet + 500 U phytase/kg.

[3]Provides per kilogram of diet: vitamin A, 10,000 IU; cholecalciferol, 2,000 IU; vitamin E, 0.25 IU; vitamin K_3, 2 mg; vitamin B_{12}, 10 μg; choline, 250 mg; folacin, 1 mg; niacin, 30 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; riboflavin, 6 mg; thiamin, 2 mg; ethoxyquin, 125 mg; Co, 0.3 mg; Cu, 10 mg; Fe, 60 mg; I, 0.5 mg; Mn, 40 mg; Se, 0.2 mg; Zn, 50 mg.

[4]Provided by BASF Korea Ltd., Seoul, Korea 100-611.

[5]DM basis.

MATERIALS AND METHODS

Experimental Diet

Diets were formulated to have the same nutrient density except for P (Table 1). Four experimental diets are as follows: T1, corn-soybean control diet [1.4% tricalcium phosphate (TCP)]; T2, control diet + phytase; T3, 0.7% TCP diet + phytase; and T4, 0% TCP diet + phytase. Natuphos®[2] was supplemented at a level of 500 U/kg of the diets (T2 to T4).

Feeding Regimen

Nine hundred and sixty 18-wk-old ISA Brown® pullets were assigned to four dietary treatments. Each treatment consisted of 12 replications of 10 cages (two birds per cage). Following 2 wk of on the prelay diet (18 and 19 wk of age), birds were given experimental diets for 21 wk. Diets in mash form and water were provided for *ad libitum* consumption. The house for the birds was provided with programmed lighting and ventilation. The lighting program started with 1430 h of light at the initiation of experiment and was increased by 30 min/wk until 1600 h of light was achieved.

Production Performance Variables and Egg Quality

The number of eggs and egg weight were recorded daily during 21 to 40 wk of age. Feed consumption was measured weekly. Random samples of 25 eggs from each treatment were collected weekly to measure egg quality such as specific gravity, eggshell strength, Haugh unit, and eggshell thickness. Specific gravity of eggs was determined every week by using the saline flotation method of Hempe *et al.* (1988). Salt solutions were made in incremental concentrations of 0.005 in the range from 1.065 to 1.120. Eggshell strength, breaking force from the blunt end to the sharp end in pounds per square inch, was measured by using The Compression Test Cell (Model TC-1) in Texture Test Systems.[3] Haugh units were calculated using the HU formula (Eisen *et al.*, 1962) based on the

[2]BASF Korea, Ltd., Seoul, Korea 100-611.

[3]Model T2100C, Food Technology Corp., Rockville, MD 20852.

TABLE 2. Effect of supplemental phytase on productivity of laying hens fed experimental diets from 21 to 40 wk of age[1]

Variables	Treatments[2] 1	2	3	4	SEM
Egg production, % hen-d	84.49[b]	86.31[a]	84.85[b]	84.83[b]	0.29
Egg production, % hen-housed	83.26[b]	85.54[a]	83.55[b]	83.71[b]	0.34
Egg weight, g	58.55[ab]	58.72[a]	58.48[b]	58.69[a]	0.07
Feed consumption, g/hen/d	112.4[c]	114.8[a]	113.5[b]	113.7[b]	0.3
Feed conversion,[3] feed:egg mass, g:g	2.25	2.24	2.26	2.26	0.015
Mortality,[3] %	2.92	1.67	3.33	3.33	0.57

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

[1]Twelve replications per treatment, 10 cages per replication, 2 hens per cage (n = 240 for each mean).

[2]T1 = control diet (1.4% TCP), T2 = control diet + 500 U phytase/kg, T3 = 0.7% TCP diet + 500 U phytase/kg, T4 = 0% TCP diet + 500 U phytase/kg.

[3]n = 12 for each mean [egg mass = (hen-day egg production × egg weight)/100].

height of albumen determined by a micrometer[4] and egg weight. Shell thickness was a mean value of measurements at three locations on the egg (air cell, equator, and sharp end) by using a dial pipe gauge.[5]

Retention and Excretion of Nutrients

After the conclusion of the feeding trial, six birds per treatment were randomly assigned to metabolic cages to determine retention and excretion of dietary nutrients. Nutrient retention was the amount of nutrient retained per hen per day, which was calculated based on the availability of nutrient and feed intake. Excreta of layers were totally collected for 3 d. Diets and excreta were analyzed by chemical procedure (AOAC, 1990) for proximate components. To determine their contents of Ca, P, Mg, Zn, Fe, and Cu, samples of feeds and excreta were dry-ashed (AOAC, 1990) and concentrations of minerals were measured at specific wavelengths for each element (Ca, 317.933; P, 214.914; Mg, 279.079; Fe, 259.940; Zn, 213,856; and Cu, 324.754 nm) by using an ICP (Inductively Coupled Plasma) Emission Spectrometer.[6] Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions[7] of each element.

Statistical Analysis

Data were analyzed by a split block design to adjust effect of production period using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1988). Significant differences among treatment means were determined at $P < 0.05$ by Duncan's new multiple range test (Duncan, 1955).

[4]Model S-8400, AMES, Waltham, MA 02254.
[5]Model 7360, Mitutoyo Corp., Kawasaki, Japan, 213.
[6]Model JY-24, Jobin Yvon, Longjumeau, Cedex, France, 91165.
[7]Junsei Chemical Co., Ltd., Tokyo, Japan.

RESULTS

Production Performance

The results obtained from the feeding trial are shown in Table 2. Enzyme supplementation to the control diet increased hen-day egg production by 2.15% and hen housed egg production by 2.74%, respectively. Egg production of the low P diets supplemented with enzyme (T3 and T4) were not significantly different from that of the control (T1). Average egg weights were significantly different among treatments, T2 being highest and T3 being lowest (Table 2). Although the average feed consumption of T2 was significantly higher (approximately 1 to 2%) than other treatments, this feed conversion ratio was not significantly different from those of the other treatments. Mortality was not significantly different among treatments.

Egg Quality

There were significant differences among treatments in the specific gravity and eggshell thickness of eggs (Table 3). Diet T1 showed the highest values, followed by T2; however, eggshell strength and broken egg:total egg ratio were not significantly different among treatments. Haugh units were not significantly different among treatments.

Retention and Excretion of Nutrients

Retention of dry matter, fat, ash, Ca, Mg, Fe, and Zn were significantly greater in phytase-supplemented groups than T1 (Table 4). Phosphorus retention of T2 was greater than T1, and those of T3 and T4 were not significantly different from that of T1. Retention of N and Cu in T2, T3, and T4 were not significantly different from that of the control. Excretion of ash, P, and Cu were lesser in phytase supplemented groups than the control. Phosphorus excretion of T4 was 41% less than the control.

TABLE 3. Quality of eggs laid by hens fed experimental diets from 21 to 40 wk of age

Variables	Treatments[1,2] 1	2	3	4	SEM
Specific gravity	1.0901[a]	1.0897[a]	1.0891[b]	1.0890[b]	0.0002
Eggshell thickness, μm	361.0[a]	359.8[ab]	357.1[b]	358.4[ab]	1.03
Eggshell strength, psi	10.05	10.10	10.21	10.22	0.076
Broken egg ratio,[3] %	0.43	0.46	0.43	0.54	0.082
Haugh unit	94.20	94.49	93.74	94.47	0.31

[a,b]Means in a row with no common superscript differ significantly ($P < 0.05$).

[1]Means of 500 eggs (25 eggs per wk × 20 wk) per treatment.

[2]T1 = control diet (1.4% TCP), T2 = control diet + 500 U phytase / kg, T3 = 0.7% TCP diet + 500 U phytase / kg, T4 = 0% TCP diet + 500 U phytase / kg.

[3]No. broken eggs: total eggs.

DISCUSSION

This experiment has shown that the egg production of hens fed low NPP (0.24 or 0.12%) diets with 500 U of microbial phytase / kg diet were not significantly different from those of the control, which contained a commercially recommended high level of NPP (0.37%). Gordon and Roland (1997) reported that feeding 0.1% NPP diet decreased egg production and feed consumption compared to 0.2 to 0.5 NPP diet but 0.1% NPP diet supplemented with 300 U of phytase / kg diet completely corrected the adverse effects. There were no significant differences among 0.2 to 0.5% NPP diets and supplementation of phytase to 0.2 to 0.5% NPP diets gave no further improvement in the performance.

These results are somewhat different from the results of Vandepopuliere and Lyons (1992), who reported that 0.2% NPP diet depressed performance of layers compared to those of 0.3 to 0.5% NPP diets. In the present experiment, however, the supplementation of 500 U of phytase / kg diet to the control diet (0.37% NPP) improved hen productivity. As the control diet contained enough NPP relative to the recommended level of 0.27% NPP (NRC ,1994), the results indicates that phytase supplementation may influence utilization of not only phytate P but also other nutrients.

The results of a metabolic trial showed that retention of major nutrients and minerals, as well as P, are generally improved. Yi *et al.* (1996a) also reported that dietary phytase supplementation improved N and

TABLE 4. Nutrient retention and excretion in laying hens fed the experimental diets[1]

Dietary component	Treatments[2] 1	2	3	4	SEM
	(g / hen / d, DM)				
Retention					
Dry matter	77.34[b]	93.96[ab]	98.17[ab]	105.82[a]	6.50
Fat	5.43[b]	6.75[a]	6.97[a]	7.17[a]	0.406
Fiber	0.73[ab]	0.61[b]	1.12[a]	0.47[b]	0.135
Nitrogen	1.99	2.41	2.67	2.70	0.223
Ash	7.54[b]	11.06[a]	10.78[a]	10.63[a]	0.786
Calcium	2.50[b]	3.30[ab]	3.05[ab]	3.49[a]	0.276
Phosphorus	0.25[b]	0.38[a]	0.31[ab]	0.21[b]	0.034
Magnesium	0.19[b]	0.25[ab]	0.25[ab]	0.28[a]	0.025
	(mg / hen / d, DM)				
Iron	12.96[b]	13.98[b]	17.53[ab]	19.43[a]	1.599
Zinc	1.43[b]	1.82[ab]	2.59[a]	2.45[a]	0.243
Copper	0.40	0.66	0.67	0.98	0.179
	(g / hen / d, DM)				
Excretion					
Nitrogen	1.53	1.52	1.36	1.43	0.092
Ash	10.26[a]	8.59[b]	8.94[b]	9.34[ab]	0.321
Calcium	2.47	2.30	2.57	2.30	0.138
Phosphorus	0.61[a]	0.54[b]	0.43[c]	0.36[d]	0.018
Magnesium	0.29	0.27	0.27	0.26	0.013
	(mg / hen / d, DM)				
Iron	31.31[ab]	33.32[a]	30.29[ab]	28.98[b]	0.982
Zinc	10.62[a]	9.84[ab]	8.74[b]	9.07[b]	0.355
Copper	2.48	2.33	2.31	2.15	0.151

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

[1]Means of six hens per treatment.

[2]T1 = control diet (1.4% TCP), T2 = control diet + 500 U phytase / kg, T3 = 0.7% TCP diet + 500 U phytase kg, T4 = 0% TCP diet + 500 U phytase / kg.

amino acids digestibility in turkey poults. It has been hypothesized that positively charged proteins can form insoluble complexes with negatively charged phytate at low pH (Cheryan, 1980; Reddy *et al*., 1982) or the binding is mediated by polyvalent cations, such as Ca, Mg, or Zn at high pH (Anderson, 1985). Protein and phytate complex has low digestibility (Thompson and Serraino, 1986) and phytase supplementation may improve protein and mineral digestibility (Yi *et al*., 1996b; Selle, 1997).

The results on egg quality of the present experiment are not conclusive. Vandepopuliere and Lyons (1992) reported that egg specific gravity was inversely related to the level of dietary NPP and egg weight. The interaction of dietary NPP level and phytase supplementation should be further studied.

Results from our metabolic trial showed that retention of crude ash was significantly increased by phytase supplementation (Table 4). This result indicates that mineral components became more available with phytase supplementation. As expected, availability of P and Zn, and retention of Ca, P, Mg, Fe, and Zn were significantly increased by supplementation of phytase, whereas excretion of crude ash, P and Zn were significantly reduced. Reduction of P excretion is particularly important in relation to the pollution potential of the hens' manure. In this experiment, the reduction of P excretion was 41% (0.61 vs 0.36 g per hen per d) with the low P diet and supplementary phytase. Simons *et al*. (1992) reported that supplemental phytase could lower by more than 40% of P excretion by increasing P availability.

It can be concluded that supplementation of 500 U of microbial phytase/kg diet can reduce the NPP level to 0.12% in the layer diet without affecting laying performance and resulting in a significant reduction of P excretion.

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