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Effect of superdosing phytase on productive performance and egg quality in laying hens

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Objective: An experiment was conducted to determine the effect of superdosing phytase on productive performance and egg quality in laying hens.
Methods: A total of 200 42-wk-old Hy-Line Brown laying hens were allotted into 1 of 5 dietary treatments with 5 replicates consisting of 8 hens per replicate. The positive control (PC) and negative control diets (NC) were prepared based on the recommended P levels in layer diets. Supplemental phytase was added to the negative control diet at 10,000 (SD10), 20,000 (SD20), or 30,000 (SD30) fytase units (FTU)/kg. Productive performance was summarized for 6 weeks from 42 weeks to 47 weeks of age. Egg quality was assessed from 4 eggs per replicate randomly collected at the conclusion of the experiment.
Results: The SD20 treatment had greater ($p<0.05$) hen-day egg production than PC, NC, and SD10 treatment groups. There was no difference in hen-day egg production between SD20 and SD30 treatment groups. However, SD30 treatment had greater ($p<0.05$) hen-day egg production than PC treatment, but showed no difference in hen-day egg production as compared to NC and SD10 treatment groups. However, egg weight, egg mass, feed intake, and feed conversion ratio were not affected by dietary treatments. Egg quality including eggshell strength, eggshell color, egg yolk color, and haugh unit was not influenced by dietary treatments.
Conclusion: Superdosing level of 20,000 FTU/kg phytase in diets has a positive effect on egg production rate, but no beneficial effect on egg quality in laying hens.

Keywords: Phytase; Egg Quality; Laying Hen; Productive Performance; Superdose

INTRODUCTION

Phosphorus (P) is one of the most important and expensive essential minerals for poultry. In cereal grain-based diets, however, 50% to 85% of P is known to be present in a phytate-form that is poorly utilized by poultry [1]. This poor utilization of phytate-P has been a concern in terms of environmental and economic aspects. Phytase is a digestive enzyme catalyzing the release of P from the phytate complex, which increases P utilization in diets [2,3]. In addition, phytase also increases utilization of other nutrients including amino acids and various cations such as Ca, Fe, Mg, K, and Mg [2,3]. Therefore, phytase is used extensively in poultry diets to date.

Some studies suggested that the recommended inclusion level of phytase in poultry diets is 500 fytase units (FTU)/kg [1,4]. However, the use of higher levels of phytase in animal diets recently has gained increasing attention, especially for poultry diets. It is reported that more than 1,000 FTU/kg phytase improved P and other nutrient utilization in diets fed to broiler chickens as compared to the recommended levels [5]. However, limited information for laying hens fed diets containing very high dose of phytase has been available although Ca and P utilization in laying hens are likely more important than in broiler chickens. To our knowledge, moreover, no

experiments have been performed to investigate the effect of very high dose of dietary phytase on egg quality in laying hens.

The objective of this experiment, therefore, was to determine the effect of superdosing phytase on productive performance and egg quality in laying hens.

MATERIALS AND METHODS

Birds, diets and experimental design

A total of 200 42-wk-old Hy-Line Brown laying hens were allotted into 1 of 5 dietary treatments with 5 replicates in a completely randomized design. Each replicate had 4 consecutive cages and each cage had 2 hens. The positive control diet (PC) and negative control diet (NC) were formulated to contain 0.38% and 0.26% non-phytate P, respectively. The concentrations of Ca in those diets were maintained at 3.91%. All other nutrients and energy were formulated to meet or exceed the NRC [6] requirement estimates for laying hens (Table 1). Then, the supplemental phytase (Phyzyme XP 10000 TPT; declared potency of 10,000,000 FTU/kg, Danisco Animal Nutrition, Marlborough, UK) was added to the NC diet at 10,000 FTU/kg (SD10), 20,000 FTU/kg (SD20), or 30,000 FTU/kg (SD30). According to the official standard measurement for phytase activity, one phytase unit (FTU) was defined as the amount of phytase that catalyzes the release of 1 μmol of inorganic P per minute from 5.1 mmol/L sodium phytate in pH 5.5 buffer at 37°C [7]. The experimental diets were given to the hens on an *ad libitum* basis for 6 weeks. A 16-h lighting schedule was used throughout experiment. The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University.

Sample collection and egg quality analysis

Detailed procedures for sample collection and egg quality analysis were described by Shin et al [8]. In short, productive performance including hen-day egg production, egg weight, egg mass, and broken and shell-less egg production rate was recorded daily. Feed intake (FI) and feed conversion ratio (FCR) were recorded weekly. The data for productive performance were then summarized for 6 weeks from 42 weeks to 47 weeks of age. Egg quality was assessed with four eggs per replicate randomly collected at the conclusion of the experiment. Eggshell strength was determined using the texture analyzer (model TAHDi 500, Stable Micro System, Godalming, UK) and was displayed as unit of compression force exposed to unit eggshell surface area. Eggshell thickness was measured at three different regions (i.e., top, middle, and bottom) using a dial pipe gauge (model 7360, Mitutoyo Co., Ltd., Kawasaki, Japan). Eggshell color was determined using the eggshell color fan (Samyangsa, Kangwon-do, Republic of Korea). Egg yolk color was estimated by the Roche color fan (Hoffman-La Roche Ltd., Basel, Switzerland). The haugh units (HU) were measured using the micrometer (model S-8400, Ames, Waltham, MA, USA), and the HU values were calculated from egg weight (W) and albumen height (H) based from the equation: $HU = 100 \log (H - 1.7W^{0.37} + 7.6)$ as demonstrated by Eisen et al [9].

Table 1. Composition and nutrient content of the experimental diets

Items (%, unless noted)	Dietary treatments[1]				
	NC	PC	SD10	SD20	SD30
Ingredients					
Corn	59.60	59.60	59.60	59.60	59.60
Soybean meal (46% CP)	25.00	25.00	25.00	25.00	25.00
Tallow	2.00	2.00	2.00	2.00	2.00
Phytase[2]	0.00	0.00	0.10	0.20	0.30
Monodicalcium phosphate	0.47	1.02	0.47	0.47	0.47
Limestone	10.05	9.82	10.05	10.05	10.05
Celite	1.00	0.68	0.90	0.90	0.90
Cornstarch	1.00	1.00	1.00	1.00	1.00
Salt	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.27	0.27	0.27	0.27	0.27
L-lysine HCl	0.08	0.08	0.08	0.08	0.08
L-threonine	0.08	0.08	0.08	0.08	0.08
Choline	0.05	0.05	0.05	0.05	0.05
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.10	0.10	0.10	0.10	0.10
Mineral premix[4]	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Nutrient content[5]					
AMEn (kcal/kg)	2,772	2,772	2,772	2,772	2,772
Crude protein	16.13	16.13	16.13	16.13	16.13
Lysine	0.80	0.80	0.80	0.80	0.80
Methionine+cysteine	0.71	0.71	0.71	0.71	0.71
Ca	3.91	3.91	3.91	3.91	3.91
Non-phytate P	0.26	0.38	0.26	0.26	0.26

CP, crude protein; AME_n, nitrogen-corrected apparent metabolizable energy; FTU, fytase units.

[1] NC, negative control (0.26% non-phytate P); PC, positive control (0.38% non-phytate P); SD10, NC+10,000 FTU/kg; SD20, NC+20,000 FTU/kg; SD30, NC+30,000 FTU/kg.

[2] Phyzyme XP (Danisco Animal Nutrition, Marlborough, UK).

[3] Provided per kg of the complete diet: vitamin A, 13,000 IU (retinyl acetate); vitamin D_3, 5,000 IU; vitamin E, 80 IU (DL-alpha-tocopheryl acetate); vitamin K_3, 4.0 mg (menadione dimethpyrimidinol); vitamin B_1, 4.0 mg; vitamin B_2, 10.00 mg; vitamin B_6, 6.0 mg; vitamin B_{12}, 20 μg; folic acid, 2.0 mg; biotin, 200 μg; niacin, 60 mg; calcium-pantothenate, 20 mg.

[4] Provided per kg of the complete diet: iron, 50 mg ($FeSO_4$); zinc, 60 mg ($ZnSO_4$); manganese, 50.0 mg (MnO); copper, 6.00 mg ($CuSO_4$); cobalt, 250 μg ($CoSO_4$); selenium, 150 μg (Na_2SeO_3); iodine, 1 mg [$Ca(IO_3)_2$].

[5] Calculated values [6].

Statistical analysis

All data were analyzed by analysis of variance as a completely randomized design using the PROC MIXED procedure (SAS Institute Inc., Cary, NC, USA). Each replicate was considered an experimental unit in the analysis of productive performance and egg quality. Outlier data were examined according to the method of Steel et al [10], using the UNIVARIATE procedure of SAS; however, no outliers were identified. The LSMEANS procedure was used to calculate treatment means and the PDIFF option of SAS was used to separate the means if the difference was significant. Significance for statistical tests was set at $p<0.05$.

RESULTS

During 6 weeks of feeding trial, SD20 treatment had a greater ($p<0.05$) hen-day egg production than NC, PC, and SD10 treatment groups. The SD30 treatment had similar egg production rate to SD20 treatment. However, SD30 treatment had greater ($p<0.05$) hen-day egg production than PC treatment, but showed no difference in hen-day egg production as compared to NC and SD10 treatment groups (Table 2). Egg weight, egg mass, broken and shell-less egg production rate, FI, and FCR were not affected by dietary treatments. Egg quality including eggshell strength, eggshell thickness, eggshell color, egg yolk color, and HU was not affected by dietary treatments (Table 3).

DISCUSSION

In the current poultry industry, phytase is commonly added to poultry diets to improve P utilization, which leads to a decrease in feed cost and P excretion in the environment [7]. Moreover, it is well-documented that dietary phytase has positive effects on productive performances of poultry [1]. The benefits result from the fact that phytase increases utilization of P and other nutrients such as Ca and amino acids [11]. The level of 500 FTU/kg phytase is recommended for most of poultry and pig diets [11]. It is reported, however, that less than 50% phytate P in diets is hydrolyzed at the level of 500 FTU/kg phytase in diets [7]; hence, some attention on the use of unconventionally high dose (i.e., superdose) of phytase has currently gained attention in poultry and pig diets [12,13].

In broiler experiments regarding effects of superdosing phytase, Shirley and Edwards [14] reported that superdosing phytase up to a level of 12,000 FTU/kg in P-deficient diets can maximize the utilization and release of P from phytate in diets. Cowieson et al [5] also reported that the levels of 24,000 FTU/kg phytase in diets improved toe ash percentage and utilization of several nutrients. In the study of Augspurger and Baker [15], the levels of 10,000 FTU/kg phytase in P and amino acid-deficient diets for 8-d old chicks maximized phytate-P utilization. The mode of actions for these beneficial effects of superdosing phytase has been associated with more liberated available P from phytate-P, which can decrease its anti-nutritional effect and generate *myo*-inositol showing vitamin like or lipotropic effects [12]. This increased utilization of phytate-P may further improve the utilization of energy and other nutrients such as amino acids and

Table 2. Effect of superdosing phytase on productive performance in laying hens[1)]

Items	Dietary treatments[2)]					SEM	p-value
	NC	PC	SD10	SD20	SD30		
Hen-day egg production (%)	94.4^{bc}	93.6^{c}	94.9^{bc}	97.3^{a}	96.6^{ab}	0.85	0.02
Egg weight (g)	68	68	67	68	66	0.7	0.22
Egg mass (g/d)	65	64	63	67	65	1.1	0.27
Broken and shell-less egg production (%)	0.03	0.02	0.02	0.03	0.06	0.013	0.33
Feed intake (g/d)	132	128	132	132	132	2.7	0.78
Feed conversion ratio (g of feed/g of egg)	2.03	2.02	2.09	1.99	2.05	0.039	0.44

SEM, standard error of the mean; FTU, fytase units.
1) Data are least squares means of 5 observations per treatment.
2) Dietary treatments = NC, negative control (0.26% non-phytate P); PC, positive control (0.38% non-phytate P); SD10, NC+10,000 FTU/kg; SD20, NC+20,000 FTU/kg; SD30, NC+30,000 FTU/kg.
a-c Means with different superscripts within a row are different ($p<0.05$).

Table 3. Effect of superdosing phytase on egg quality in laying hens[1)]

Items	Dietary treatments[2)]					SEM	p-value
	NC	PC	SD10	SD20	SD30		
Eggshell strength (kg/cm^2)	3.4	3.2	3.2	3.1	3.2	0.16	0.65
Eggshell thickness (μm)	436	428	445	433	433	5.40	0.15
Eggshell color (Color fan)	11.8	10.7	11.2	11.4	11.0	0.39	0.33
Eggshell color (Hunter color)							
L*	53.0	53.0	53.7	53.1	54.2	0.59	0.50
a*	19.7	20.0	19.7	19.3	19.0	0.40	0.24
b*	25.4	25.3	25.6	25.0	24.9	0.30	0.40
Egg yolk color (Roche color fan)	7.8	7.4	7.6	8.0	7.2	0.30	0.26
Haugh unit	96.7	97.0	98.1	97.5	98.7	1.06	0.45

SEM, standard error of the mean; FTU, fytase units.
1) Data are least squares means of 5 observations per treatment.
2) NC, negative control (0.26% non-phytate P); PC, positive control (0.38% non-phytate P); SD10, NC+10,000 FTU/kg; SD20, NC+20,000 FTU/kg; SD30, NC+30,000 FTU/kg.

minerals in diets, which is known as an extra-phosphoric effect of phytase [12,16].

As compared to broiler chickens, however, a scarcity of data regarding effects of superdosing phytase has been reported in laying hens although Ca and P utilization for laying hens are likely more important than for broiler chickens. Augspurger et al [17] reported that the levels of 10,000 FTU/kg phytase in diets had no benefits on productive performance in laying hens. Meyer and Parsons [18] also found no improvement in productive performance by feeding diets containing up to 15,000 FTU/kg phytase to laying hens. Similarly, in the current experiment, there were no beneficial effects on productive performance in laying hens when up to 10,000 FTU/kg phytase was added to diets. However, we found significant improvements in egg production rate at the inclusion levels of 20,000 FTU/kg phytase, which was greater than the inclusion levels of previous experiments; however, no further improvements were observed at the inclusion levels of 30,000 FTU/kg phytase in this experiment. These variable results among experiments may be associated with differences in experimental designs such as animal genetics, age of hens, feeding duration, inclusion levels, sources of phytase, and environment [7]. In addition, it is suggested that there is an optimal ratio of Ca to available P in diets for maximizing egg production in laying hens due to their antagonistic effects [6]. In the present experiment, the experimental diets (NC, SD10, SD20, and SD30) were formulated with less amounts of available P than the recommended amounts but contained the recommended amounts of Ca [19], and accordingly, the ratio of Ca to available P in diets was greater than the recommended ratio. Thus, we speculated that inclusion of 20,000 FTU/kg phytase in diets may restore the balance of Ca and available P by increasing P utilization, which likely lead to an improvement in egg production rate. Similar improvements have been often observed for poultry when diets contained the recommended levels of Ca but low level of available P [7,19]. However, it appeared that inclusion of 10,000 or 30,000 FTU/kg phytase in diets could not induce the optimal balance of Ca and available P for laying hens used in this experiment.

No improvement in egg quality as observed in this experiment was unexpected because increased Ca and P utilization by dietary phytase have been reported to improve egg quality, especially for eggshell strength and thickness [20,21]. To our best knowledge, there are no data pertaining to effects of superdosing phytase on egg quality in laying hens, and therefore, it was difficult to find the clear reason for our observation. It may be speculated that, based on the observation for similar FI with increasing phytase levels in diets, P and Ca concentrations in diets were sufficient support the proper eggshell formation in the current experiment.

It can be concluded, therefore, that the superdosing level of 20,000 FTU/kg phytase in diets has a positive effect on egg production rate, but no beneficial effect on egg quality in laying hens.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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