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≪Research Note≫

Effects of Microbial Phytase Supplementation on Egg Production and Egg Quality in Hy-line Brown Hens During the Late Laying Period

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The objective of this study was to evaluate the effects of microbial phytase on egg production and egg quality in older hens. A total of 216, 63-week-old Hy-line brown laying hens were distributed in a randomized complete design 10-week feeding trial of 3 dietary treatments with 12 replications per treatment and 6 hens per replication. The 3 dietary treatments were corn-soybean meal-based diets supplemented with 0% (CON), 0.06% (TRT1), and 0.12% (TRT2) microbial phytase. Significantly higher hen-day egg production was observed in the TRT1 treatment compared to CON ($P<0.05$), except during the first two weeks of the experiment. During weeks 3, 4, and 9, TRT2 had a greater hen-day egg production percentage than CON ($P<0.05$). The damaged egg ratio was not affected. The egg quality parameters (e.g., eggshell color, eggshell strength, albumen height, egg weight, and the Haugh unit) were affected by microbial phytase supplementation ($P<0.05$). However, there were no significant effects on the eggshell thickness and yolk color. In conclusion, microbial phytase supplementation to the diets of older hens could improve production performance, extend the peak laying period, and alter the egg quality parameters.

Key words: egg quality, microbial phytase, older hens, production performance

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Introduction

It is now commonplace to supplement poultry diets with feed enzymes to enhance nutrient utilization and performance by counteracting the negative influences of targeted substrates. Phytate (myo-inositol hexakisphosphate) is the main form of stored phosphorus (P) in plant-derived feedstuffs such as cereals, legumes, and oilseeds (Viveros *et al*., 2000; Lichtenberg *et al*., 2011). However, this P form is poorly available to poultry because of inefficient dephosphorylation via endogenous phytase activity in the gastrointestinal tract, especially in hens during late laying period (Torrallardona *et al*., 2012). Older hens are also more sensitive to P deficiency, so the total and available P levels in the diet are kept high for laying hens in the late laying period (Boling *et al*., 2000). Therefore, the dietary inclusion of high doses of exogenous phytase may be an effective way to combat P deficiency in the late laying period.

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The phytase enzyme catalyzes the hydrolysis of phytate and releases a usable form of inorganic P to poultry (Mullaney *et al*., 2000). It is produced by plants, animals, and microorganisms, and microbial phytase is a commonly used exogenous enzyme in non-ruminant animals (Musilová *et al*., 2014). The poultry gastrointestinal tract lacks adequate amounts of endogenous phytase to hydrolyze phytate and release bound P (Sebastian *et al*., 1998), so their diets are usually supplemented with inorganic P. This supplementation is expensive and fails to address the issue of over-supplementation, which can lead to environmental P pollution in the soil and groundwater. For example, the manure-based excretion of excess P in areas of concentrated animal production poses an environmental threat (Ravindran *et al*., 1998). Economic and environmental concerns have generated renewed interest in phytase use to reduce the reliance on inorganic P supplements and to improve P utilization in feedstuffs.

Generally, the performance and egg quality of hens in the late laying period decreases, but these negative effects of age can be mitigated by nutrition. Phytase diet supplementation improved the feed intake, egg production, and egg quality of laying hens (Gordon and Roland, 1997; Kozlowski and Jeroch, 2011; Englmaierova *et al*., 2015; Englmaierová *et al*., 2017). However, research on higher dose microbial phytase diet supplementation is limited (Zaghari *et al*., 2008; Deniz *et al*., 2013). Therefore, the objective of the present study was to determine the effects of microbial phytase on egg production and egg quality in older hens (63 to 72 weeks) fed corn-soybean meal-based diets.

Materials and Methods

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University, South Korea.

Phytase Source

The phytase used in this study was the commercial preparation HiPhos (GT) phytase (Ronozyme®, DSM Nutritional Products, Basel, Switzerland). The active agent of the product is *6-phytase* (EC 3.1.3.26), derived from *Citrobacter braakii* bacteria expressed in the strain *Aspergillus oryzae* (DSM 22594). The product has a guaranteed minimum *6-phytase* activity of 10 000 phytase units (FYT) per gram. One FYT is defined as the amount of phytase required to release 1 μmol of inorganic P per min from a 5 mM solution of sodium phytate at pH 5.5 and 37℃. The activity of phytase used in this experiment is 10 000 FYT/g.

Birds, Diets, Experimental Design, and Housing

Hy-line brown laying hens (n=216; 63 weeks old) were randomly assigned to 1 of 3 treatments in a 10-week experiment, with 12 replications and 6 hens per replication (1 hen/cage). The 3 dietary treatments were corn-soybean meal-based diets supplemented with 0%, 0.06% (6000 FYT/kg), or 0.12% (12 000 FTY/kg) phytase. The basal diet was formulated according to the recommendations of the Hy-line brown Management Guide (2014); the composition of the basal diet is shown in Table 1. To ensure complete enzyme mixture in the diets, premix processing was conducted according to the methods of Liu *et al*. (2019). The hens were individually housed in cages (38 cm width×50 cm length×40 cm height) in a windowless and climate-controlled room at 23℃, with sixteen hours (05:00 to 21:00) of artificial light per day. Feed and water were provided *ad libitum*, and all of the diets were presented in mash form.

Production Performance and Egg Quality Parameters

The number and weight of the laid eggs were recorded daily, per replication, and egg production was expressed as the average hen-day production. The collected eggs were classified as normal or damaged (i.e., broken eggs, cracked eggs, and shell-less eggs) to calculate the damaged egg ratio. At 3-week intervals and the conclusion of the experiment, 36 eggs (3 eggs per replication, excluding the damaged eggs) were randomly collected from each treatment at 17:00 to determine the egg quality at 20:00 the same day. The eggshell breaking strength was evaluated via an eggshell force gauge model II (Robotmation Co., Ltd., Tokyo, Japan), and a dial pipe gauge (Ozaki MFG. Co., Ltd., Tokyo, Japan) was used to measure eggshell thickness – calculated as the average thickness of the rounded end, pointed end, and middle of the egg, excluding the inner membrane. An eggshell color fan (DSM, Basel, Switzerland) was used to visually score the eggshell color. Finally, the yolk color, albumen height, and Haugh unit (HU) were evaluated on an egg multi-tester machine (Touhoku Rhythm. Co., Ltd., Fukushima, Japan).

Table 1. **Ingredient and nutrient compositions of the basal laying hen diet (as-fed basis)**

Item	
Ingredients (%)	
Corn	52.71
Soybean meal	10.99
Sesame meal	2
Distillers dried grains with soluble	20.01
Palm kernel meal	1.85
Tallow	0.7
Limestone	11.01
Mono-di-calcium phosphate	0.06
Salt	0.05
Methionine	0.05
Lysine	0.27
Choline	0.1
Vitamin premix[1]	0.1
Mineral premix[2]	0.1
Nutrient composition (%)	
Metabolizable energy (kcal/kg)	2770
Dry matter	89.28
Moisture	10.72
Crude protein	15.70
Crude fat	4.01
Crude fiber	3.09
Crude ash	14.45
Calcium	4.31
Total phosphorus	0.37
Non-phytase phosphorus	0.12
Total lysine	0.76
Total methionine	0.38
Total cysteine	0.27
Total threonine	0.58
Total tryptophan	0.16

[1] Provided per kg of complete diet: 11 025 IU vitamin A; 1103 IU vitamin D_3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 μg vitamin B_{12}.
[2] Provided per kg of complete diet: 12 mg Cu (as Copper sulfate pentahydrate); 85 mg Zn (as Zinc sulphate); 8 mg Mn (as Manganese dioxide); 0.28 mg I (as Potassium iodide); 0.15 mg Se (as Sodium thiosulfate).

Statistical Analysis

The data were analyzed as a randomized complete design using the General Linear Models (GLM) procedure in SAS (SAS Institute, Cary, NC, USA). The replications (n=12) served as the experimental unit. Differences between the treatments were detected by Tukey's multiple range test, and the results are presented as means and pooled standard error of the means (SEM). Probability values less than 0.05 were considered significant.

Results and Discussion

Productive Performance

The effects of microbial phytase on the production performance of older hens are presented in Table 2. Throughout the experiment, except for the first two weeks, higher hen-

Table 2. **Effects of microbial phytase dietary supplementation on the production performance in older hens**[1]

Items	CON	TRT1	TRT2	SEM[2]	P-value
Hen-day egg production (%)					
Week 1	82.34	82.32	81.92	0.4495	0.1795
Week 2	83.94	85.52	85.12	0.7024	0.4092
Week 3	81.55[b]	87.30[a]	87.70[a]	1.0831	0.0040
Week 4	80.95[b]	86.90[a]	86.11[a]	0.6680	0.0002
Week 5	79.96[b]	84.92[a]	83.13[ab]	1.0259	0.0194
Week 6	82.54[b]	88.89[a]	84.92[b]	0.7769	0.0006
Week 7	84.92[b]	89.09[a]	88.10[ab]	1.0182	0.0389
Week 8	84.13[b]	90.48[a]	87.30[ab]	1.2980	0.0196
Week 9	82.74[b]	86.71[a]	86.71[a]	0.9987	0.0274
Week 10	81.35[b]	85.12[a]	83.53[ab]	0.9516	0.0542
Over all	82.24[c]	87.02[a]	85.75[b]	0.3063	<.0001
Damaged egg ratio (%)					
Week 1	0.47	0.46	0.00	0.2702	0.4016
Week 2	1.45	0.00	0.00	0.4354	0.0622
Week 3	0.75	0.24	0.24	0.5009	0.7177
Week 4	1.47	0.46	0.00	0.7438	0.3939
Week 5	0.75	0.00	0.23	0.4630	0.5276
Week 6	0.25	0.00	0.00	0.1415	0.4019
Week 7	0.71	0.00	0.00	0.2801	0.1722
Week 8	1.21	0.00	0.00	0.6973	0.4019
Week 9	0.97	0.00	0.00	0.5578	0.4019
Week 10	0.25	0.00	0.00	0.1436	0.4019
Over all	0.83	0.12	0.05	0.3944	0.3414

[1] Note: CON, basal diet; TRT1, CON + 0.06% microbial phytase; TRT2, CON + 0.12% microbial phytase. Values represent least squares means of 12 replicate cages containing 6 birds per cage.
[2] Standard error of means.
[a, b, c] Within a row, different superscripted letters are significantly different at $P<0.05$.

day egg production percentages were observed in the TRT1 group than in the CON group ($P<0.05$). During weeks 3, 4, and 9, the TRT2 treatment exhibited a greater hen-day egg production percentage than the CON group ($P<0.05$). However, the damaged egg ratio was not affected by phytase supplementation ($P>0.05$). Overall, TRT1 had the highest hen-day egg production ratio among the treatments ($P<0.05$), and TRT2 had a greater egg production than CON ($P<0.05$). Other studies have also reported that phytase dietary supplementation could affect the egg production of laying hens. Van der Klis *et al*. (1997) reported a significant increase in egg production percentage with phytase supplementation at 250 and 500 FTU/kg, and Ciftci *et al*. (2005) found that 300 and 600 U/kg microbial phytase enzyme supplementation improved the hen-day egg production of 30-week-old hens. Sari *et al*. (2012) included 0.035% (500 FYT phytase/kg feed) phytase in a non-P supplemented diet and observed enhanced egg performance at 23 to 43 weeks of age. More recently, Kim *et al*. (2017) supplemented with 20 000 FTU/kg of phytase and reported increased hen-day egg production in 42- to 47-week-old hens. Still, the effects of phytase on egg production performance remain controversial due to some conflicting reports (Jalal and Scheiderler, 2001; Liebert *et al*., 2005; De Morelos, 2011; Englmaierova *et al*., 2015). De Morelos (2011) reported no effects on daily egg production, egg numbers, egg mass, or egg weights in 35- to 47-week-old hens supplemented with 1200 IU/kg of phytase. Abbasi *et al*. (2015) also reported no effects of 600 U/kg of phytase supplementation on the egg production ratio or egg production of the hen house. Owing to the lack of available data, comparisons of the phytase supplementation responses in late laying period hens (63 to 72 weeks) is impossible. Inconsistencies in the prior results may be due to variations in the supplemental duration, dose, and sources of phytase, or the feed ingredients and age of the hens. According to the Hy-Line Brown Commercial Layers Management Guide (2016), the average egg production of 63- to 72-week-old hens is 82.2%. In this study, the egg production of the CON treatment was similar to the breeder recommendations, while the TRT1 and TRT2 treatments had significantly improved egg production. There were no nutritional limitations in the diet of the control group, suggesting that the improvements in hen-day egg production in TRT1 and TRT2 were not due to increased feed consumption but improved nutrient use by the birds. Sohail and Roland (2000) reported that 300 FTU/kg of phytase improved calcium (Ca) availability and egg specific gravity. Camden *et al*. (2001) found that the ileal digestibility of P, starch, and fat were linearly affected by the addition of 250, 500, and 1000 U/ kg of phytase to the diets. Supplementation with 500 U/kg of phytase enhanced the utilization of total P and crude protein compared to non-supplemented treatments (Yao *et al*., 2007). Li *et al*. (2014) reported that 250 U/kg phytase supplementation could improve the intestinal development of 24- to 47-week-old hens. Furthermore, recent work has highlighted the increased interest in higher levels of phytase supplementation in poultry diets. Cowieson *et al*. (2009) compared the recommended levels of supplementation to > 1000 FTU/kg of phytase and reported improved P and other nutrient utilization in broilers. Kim *et al*. (2017) super-dosed phytase (20 000 FTU/kg) and observed positive effects on the egg production rate in 42-week-old hens. Following the degradation of phytate, inorganic P and other nutrient components were obtained, indicating better digestibility and nutrient absorption. Nutrients play a crucial role in the late laying period, improving egg production and extending the peak of the laying period. The results of this study suggest positive effects of phytase supplementation on the hen-day egg production and the damaged egg ratio in the late laying period and provide new insights for phytase use in older hens.

Egg Quality

The effects of microbial phytase supplementation on the egg quality parameters in older hens are shown in Table 3. Phytase supplementation did not affect eggshell thickness and yolk color throughout the experiment ($P>0.05$). For eggshell color, TRT2 had a significantly lower score than CON and TRT1 at week 9 ($P<0.05$), whereas TRT2 had a significantly higher score than CON and TRT1 at the end of the experimental period ($P<0.05$). Eggshell strength dif-

Table 3. **Effects of microbial phytase dietary supplementation on the egg quality in older hens[1]**

Items	CON	TRT1	TRT2	SEM[2]	P-value
Week 3					
Eggshell color	11.07	11.27	11.40	0.1509	0.2982
Eggshell thickness (10^{-2} mm)	35.47	35.71	34.99	0.3335	0.3081
Eggshell strength (kg/cm^2)	3.71^a	3.63^{ab}	3.48^b	0.0649	0.0420
Albumen height (mm)	12.00^a	9.31^b	9.31^b	0.2803	<.0001
Yolk color	6.95	7.03	7.22	0.0941	0.1106
Egg weight (g)	64.34	65.94	64.15	0.5836	0.0663
Haugh unit	104.77^a	92.76^b	90.94^b	1.6385	<.0001
Week 6					
Eggshell color	10.80	10.90	11.17	0.1791	0.3334
Eggshell thickness (10^{-2} mm)	33.26	33.60	33.42	0.4065	0.8371
Eggshell strength (kg/cm^2)	3.54^a	3.25^b	3.26^b	0.544	0.0005
Albumen height (mm)	7.45	7.46	7.83	0.1667	0.1860
Yolk color	6.59	6.82	6.90	0.1259	0.2166
Egg weight (g)	63.53^a	65.58^a	60.56^b	0.6286	<.0001
Haugh unit	89.12	90.14	87.34	0.9359	0.1103
Week 9					
Eggshell color	11.37^a	11.48^a	10.50^b	0.2936	0.0013
Eggshell thickness (10^{-2} mm)	34.59	33.47	34.56	0.5368	0.2485
Eggshell strength (kg/cm^2)	3.66	3.61	3.46	0.1013	0.3536
Albumen height (mm)	7.75	7.79	7.44	0.2573	0.5736
Yolk color	6.69	6.54	6.43	0.1279	0.3781
Egg weight (g)	63.44	64.70	62.25	1.1046	0.3007
Haugh unit	89.24^{ab}	91.80^a	84.02^b	1.8650	0.0150
Week 10					
Eggshell color	10.70^b	10.67^b	11.50^a	0.1996	0.0060
Eggshell thickness (10^{-2} mm)	33.66	33.54	33.13	0.3927	0.6089
Eggshell strength (kg/cm^2)	3.45	3.61	3.42	0.0961	0.3399
Albumen height (mm)	7.77	7.72	7.68	0.2492	0.8840
Yolk color	6.64	6.56	6.56	0.1397	0.8840
Egg weight (g)	63.33	63.96	62.29	1.0409	0.5199
Haugh unit	91.01	85.72	86.80	1.7110	0.0782

[1] Note: CON, basal diet; TRT1, CON + 0.06% microbial phytase; TRT2, CON + 0.12% microbial phytase. Values represent least squares means of 12 replicate cages containing 6 birds per cage.

[2] SEM, Standard error of means.

[a, b] Within a row, different superscripted letters are significantly different at $P<0.05$.

fered significantly between TRT2 and CON at week 3 ($P<0.05$), but at week 6, the CON group had a significantly stronger eggshell than TRT1 and TRT2 ($P<0.05$). For albumen height, TRT1 and TRT2 were both lower than CON at week 3 ($P<0.05$). Additionally, the Haugh unit was significantly affected at weeks 3 and 9 ($P<0.05$). After 12 months of laying, the egg-laying ability of commercial hens starts to decline to a point where the flock is commercially unviable (Browne, 2002). The hens used in this study were from this period, which may explain the erratic results for the egg quality parameters. Kim *et al*. (2017) also reported no effects of super-dosing phytase (10 000 to 30 000 FTU/kg) on the eggshell thickness and eggshell strength of 47-week-old hens. However, Englmaierova *et al*. (2015) supplemented 38- to 52-week-old hens with 350 FTU/kg of phytase and 2.1 g/kg non-phytate P and observed high eggshell thickness values but no effects on the shell breaking strength. The eggshell protects the egg against damage and microbial contamination and prevents desiccation. The bulk of the eggshell is made of calcium carbonate crystals that are stabilized by a protein matrix, and the organic matrix is thought to play a role in calcium deposition during the mineralization process (Lavelin *et al*., 2000; Arias and Fernandez, 2001; Hunton, 2005). The effectiveness of phytase in improving the eggshell strength may be associated with the utilization of minerals. The color agent present in the diet could affect yolk color, and the yolk color is directly influenced by pigments in the feed ingredients (Park *et al*., 2018). The results of this study showed that yolk color was affected by phytase supplementation; similar results were documented by Hassanien and Sanaa (2011). Li *et al*. (2014) reported that phytase in the diet may enhance nutrient absorption via greater villus height and crypt depth, thereby improving intestinal health. Similarly, Yan *et al*. (2009) noted that the digestibility of calcium, nitrogen, and P increased linearly with increasing phytase doses in 68-week-old hens. Um *et al*. (1999) supplemented with 250 U/kg of phytase and observed significant effects on the mineral contents of the tibia (i.e., zinc, iron, copper, and magnesium). The beneficial effects on egg weight and HU may be due to favorable alterations in the intestinal environment and function, which subsequently increased intestinal nutrient absorption. However, further experiments are needed to explore the mechanisms of phytase supplementation on egg quality and to identify the optimal dose in older hens.

In conclusion, the results of this study suggest that microbial phytase supplementation to corn-soybean meal-based diets could improve the hen-day egg production, reduce the egg damage ratio, and alter the egg quality parameters in 63- to 72-week-old hens.

Conflicts of Interest

The authors declare no conflict of interest.

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