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RESEARCH ARTICLE

EFFECTS OF PHYTASE SUPPLEMENTATION ON THE PERFORMANCE AND EGG QUALITY OF FREE-RANGE LAYERS

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ABSTRACT

This study was carried out to the effects of supplementation of phytase enzyme to free-range layer diets on performance and egg quality traits. In the trial, at 48 wk old 152 Lohman Brown layers were divided into 3 dietary treatments with 2 replicates. Diets were fed 8 wk periods. Basal diets with 16, 75% crude protein and 2795 kcal/kg metabolisable energy were used. Three dietary treatments were formed as followed; (1) control group diet had 0 FTU/kg with phytase (T1), (2) T2 group diet had 250 FTU/kg phytase and (3) T3 group diet had 500 FTU/kg phytase. Feed and water were supplied for *ad-libitum*. Light was provided 16 hours daily. The results were indicated that egg production, feed intake and egg weight were significantly (P<0,01) increased by phytase addition. The treatments did not affect egg shape index, haugh unit and specific gravity. On the other hands, phytase supplementation was increased egg weight, albumen and yolk weight, egg shell weight and shell thickness. An according to result of the experiment that phytase supplementation of free-range layer diets had significant positive effects on performance and egg quality traits.

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INTRODUCTION

The poultry industry has experienced significant change in the last two decades. For many years, a relatively large number of consumers have been interested in buying free-range or organic eggs. This has led to an increase in the production of both free-range and organic poultry in many countries. These systems can be environmentally friendly, sustaining animals in good health with high welfare standards and lead to better quality products (Sundrum, 2001) and more flavoursome products (Sauveur, 1997). By eating various grasses and herbs, free-range layers enrich their food and assimilate valuable natural nutrients that influence some egg quality traits (Nys, 2000; Van den Brand *et al*., 2004). The consequences of alternative housing systems (especially outdoor systems) for egg quality are not yet clear. Free-range layer diets are based largely on grasses, cereal grains and oilseed meals. Unfortunately, approximately 2/3 of the phosphorus in these plant materials are present in the form of phytic acid (Akyurek *et al*., 2005). The ability of poultry to use phytate phosphorus is poor (Wu *et al*., 2004) due to insufficient quantities or lack of intestinal phytase secretion (Ravindran *et al*., 1998; Sebastian *et al*., 1998). This inadequacy of poulty to use phytate phosphorus results in the excretion of large amounts of phosphorus in manure, posing an environmental concern especially, in areas of intensive animal production (Wu *et al*., 2004).

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On the other hands, phosphorus is an essential nutrient in several metabolic processes and it is one of the major mineral elements required poultry. Phosphorus and calcium play a major role in the development and maintenance of skeletal system and for eggshell formation (Kannan *et al*., 2011). Phytase supplementation of the feed has been shown to improve the use of phytate phosphorus in laying hens (Gordon and Roland, 1997; Van der Klis *et al*., 1997; Carlos and Edwards, 1998; Um and Paik, 1999; Jalal and Scheideler, 2001; Scott *et al*., 2001). However the influence of phytase supplementation on performance and egg quality have not been fully studied in free-range laying hens. Therefore, the aim of the present experiment was to examine the effect of microbial phytase on performance and egg quality in free-range laying hens fed corn-soybean meal based diets.

MATERIALS AND METHODS

Hens, housing and feed

A total of 150 Lohmann Brown hens at the age of 48 weeks were distributed into three groups with two replicate flocks of 25 hens each. Each replicate flock was housed in a wooden house of 8 m^2, equipped with perches, 5 nests, 1 suspended drinker and 1 suspended feeder. The hens had 24h access to an outdoor yard of 8 m^2/bird. The each outdoor yard was also equipped with a suspender drinker. Throughout the experimental period, the birds were fed on commercial layer diet (NRC, 1994) which was given *ad-libitum*.

Light was provided 16 hours daily. The calculated contents of crude protein and metabolisable energy in the basal diet were 16.75% and 2795 kcal/kg. The composition of basal diet is shown in Table 1.

Experimental design and measurements

The experimental periods lasted 8 weeks and started at a hen age of 48 weeks and consisted of the following 3 experimental treatments as: (1) control group diet had 0 FTU/kg with phytase (T1), (2) T2 group diet had 250 FTU/kg phytase and (3) T3 group diet had 500 FTU/kg phytase. In the present experiment, Natuphos® 10,000G, (a phytase preparation obtained from *Aspergillus niger* and supplied by BASF, Turkey) was used as supplemental phytase. Egg production and egg weights were recorded daily. Feed consumption was measured weekly. Random samples of 6 eggs from each replicates were collected biweekly to measure some eggshell quality and internal egg quality such as specific gravity, eggshell strength, eggshell thickness, eggshell weight, eggshell percentage, Haugh unit, shape index, albumen and yolk weight, albumen and yolk pH.

Specific gravity of eggs was determined biweekly by using salt solutions with 0.005 incremental concentrations in the range from 1.065 to 1.120 (Hempe *et al*., 1988). Eggshell strength breaking force from blunt to sharp end in kg/cm^2, was measured by using the compression test cell. Shell thickness was the mean value of measurements made at three locations (aircell, medium, and sharp end) with the micrometer [Type D/06 20717 (0,01-0,25 µm) micrometer, Fabrikat Mauser, Germany] after determination of eggshell strength. Egg weight was measured using a balance and was recorded to the nearest 0,01 g. The height of the thick albumen and egg yolk were measured within a tripod micrometer. The Haugh units were calculated from albumen height and egg weight using Haugh unit=100log $(H+7.57-1.7W^{0.37})$, where H is the albumen height (mm) and W is the weight of the egg (g). After the eggs were broken, the albumen was separated from yolk. The pH of the albumen and yolk was measured using a pH meter (pH meter, Inolab level 1, WTWGmbH, Weilheim, Germany). Cracked eggs were determined each week on the same day by examining all the eggs collected from the flocks and they were subjected to a bulb control in the dark to identify the cracked eggs. The rate of cracked eggs was calculated as percentage of the total collected eggs on the same day.

Statistical analysis

Collected data were recorded on a weekly basis and statistically subjected to ANOVA using the PASW Statistics18 packed program for windows (PASW Statistics18, 2010). The differences between group means were evaluated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Performance: The results presented in Table 2 indicate that layer production performance was significantly affected by dietary treatments. Phytase supplementation to the control diet, 250 FTU/kg and 500 FTU/kg increased ($P<0,05$) egg production from 94,96% to 95,56% and 96,29% respectively. Egg production of hens consuming diets with phytase was also higher than that of the control diet. This result is in agreement with observations by Van der Klis *et al*. (1997) and Keshavarz (2000). In other study, Um and Paik (1999) who observed that phytase supplementation to the diet containing between 3,0 and 3,5 g aP/kg increased hen-day egg production significantly. Similarly, Lim *et al*. (2003) found that supplementation of microbial phytase (300U/kg) in the diet of laying hens can improve egg production. Significant differences in feed intake and egg weights ($P<0.01$) showed the same trends as egg production. In the absence of phytase, feed intake was significantly decreased. Similar results were reported by Punna and Roland (1999). The higher feed intake in phytase supplemented groups may be the results of higher egg production. The intake of the birds fed the diets containing the 500 FTU/kg enzyme group (125 g/d) was the highest and those following the 250 FTU/kg enzyme group (120 g/d) and control group (116,55 g/d) respectively ($P<0,01$). These differences among the groups may be due to from the use of microbial phytase enzyme amount in diets. Because, the increase of the amount of phytase enzyme in diets, the feed intake was increased too. The improved to the feed intake with phytase released P, is potential for other nutrients to show a higher availability or in the case of minerals retention. In particular, positively-changed (cationic) minerals such as calcium, zink, copper, cobalt, iron, magnesium, nickel and manganese are all known to from complexes with phytate and show higher digestibility values in the presence of phytase (Remus, 2005). In agreement with these result, in studies phytase use reported that, the addition phytase in poultry diets were increased feed intake (Punna and Roland, 1999; Um and Paik, 1999; Jalal and Scheideler, 2001).

The highest feed efficiency was determined in control group (1,77) and those following the 250 FTU/kg enzyme group (1,84) and 500 FTU/kg enzyme group (1,85) ($P<0,01$). This results does not agree with the previous reports. Liebert *et al*. (2005) found that microbial phytase significantly improved feed conversion ratio whereas Boling *et al*. (2000) reported that FCR was not affected by phytase supplementation. Similarly, in studies phytase use reported that, the addition phytase in poultry diets were increased feed efficiency (Van der Klis, 1997; Jalal and Scheideler, 2001).The highest egg weight was determined in 500 FTU/kg enzyme group (67,69g) and those following the 250 FTU/kg enzyme group (65,36g) and control group (65,77g) respectively ($P<0,01$). This results just as results of Peter (1992) indicated that laying hens fed a diet with phytase had significantly higher egg weight than hens fed on the same diet without supplemental phytase (control). Also, Ciftci *et al*. (2005) who reported that the highest egg weight was determined in 600 U of microbial phytase kg^{-1} enzyme group (70,29g) and these following the 300 U of microbial phytase kg^{-1} enzyme group (67,37 g) and control group (64,31 g) respectively ($P<0,01$). While presented results are disagreement with findings of Scott *et al*. (1999) reported that, phytase supplementation had no significant effect on egg weight. Increasing egg production as a result of phytase supplementation may be due to that phytate presented in the most ingredients of poultry diet is capable of forming complexes with essential nutrients such as proteins and some in organic cations. The use of phytase led to release these essential nutrients and improving nutritional value of poultry diet which can be positively effect on productive performance of laying hens (Panda *et al*., 2005). In addition, several studies have examined the influence of microbial phytase on protein digestion and utilization in poultry (Kornegay, 1996; Klis and Versteegh, 1996; Yi *et al*., 1996; Sebastian *et al*., 1997).

Table 1. Composition and nutrient concentrations of experimental diets

Ingredients (%)	T1	T2	T3
Maize	58,606	58,606	58,606
Soybean meal (% 46)	16,990	16,990	16,990
Sunflower meal (% 36)	12,895	12,895	12,895
Limestone	8,217	8,217	8,217
Dicalcium phosphate	1,340	1,340	1,340
Soybean oil	1,276	1,276	1,276
Common salt	0,350	0,350	0,350
Vitamin-Mineral Premix[1]	0,180	0,180	0,180
DL-Methionine	0,146	0,146	0,146
Phytase	-	0,0025	0,0050
TOTAL	100,000	100,000	100,000
Calculated nutrient concentrations			
Dry matter, %	87,780	87,780	87,780
ME, kcal/kg	2795,000	2795,000	2795,000
Crude protein, %	16,750	16,750	16,750
Ether extract, %	3,537	3,537	3,537
Linoleic acid, %	2,158	2,158	2,158
Crude fibre, %	4,000	4,000	4,000
Calcium, %	3,500	3,500	3,500
Available phosphorus, %	0,360	0,360	0,360
Sodium, %	0,186	0,186	0,186
Lysine, %	0,746	0,746	0,746
Methionine, %	0,423	0,423	0,423
Met+Cys, %	0,680	0,680	0,680

[1] Supplied per kg of diet: vit A: 12 500 000 IU, vit D_3: 2 500 000 IU, vit E: 40 000 mg, vit K_3: 4 500 mg, vit B_1: 2 000 mg, vit B_2: 7 000 mg, nichotin amide: 40 000 mg, cal-D-panth.:8 000 mg, vit B_{12}: 20 mg, folic acid: 750 mg, Mn: 100 000 mg, Fe: 35 000, Zn: 60 000, Cu: 5 000,Se: 300 mg, I: 500 mg, Co: 100 mg.

Table 2. Effects of phytase supplementation on layer production performance (48 to 56 wk of age)

Treatment	Hen-day production (%)	Feed intake (g/d)	Egg weight (g)	Feed conversion
T1	94,36^{a}	116,55^{a}	65,77^{a}	1,77^{a}
T2	95,56^{ab}	120,00^{a}	65,36^{a}	1,84^{b}
T3	96,29^{b}	125,00^{b}	67,69^{b}	1,85^{b}
SEM	0,279	0,818	0,102	0,125
P-level	0,017	0,001	0,001	0,023

Table 3. Effects of phytase supplementation on egg internal quality (48 to 56 wk of age)

Treatment	Egg weight (g)	Albumen (%)	Yolk (%)	Yolk:Albumen	Yolk weight (g)	Albumen weight (g)	HU	Albumen pH	Yolk pH
T1	65,68^{a}	58,40^{a}	25,24^{b}	0,43^{b}	16,57^{a}	38,38^{a}	90,40	8,19^{a}	6,28
T2	67,70^{b}	59,49^{b}	24,35^{a}	0,41^{a}	16,45^{a}	40,33^{b}	89,33	8,25^{a}	6,26
T3	68,30^{b}	58,86^{ab}	25,22^{b}	0,43^{b}	17,21^{b}	40,23^{b}	87,34	8,42^{b}	6,29
SEM	0,367	0,214	0,143	0,037	0,112	0,301	0,696	0,025	0,014
P-level	0,009	0,113	0,014	0,033	0,010	0,011	0,190	0,001	0,654

Table 4. Effects of phytase supplementation on egg shell quality (48 to 56 wk of age)

Treatment	Specific gravity	Shell weight (g)	Shell breaking strength (kg/cm^2)	Shell thicknes (µ)	Shape index	Shell percentage (%)	Broken and soft shell (%)
T1	1,089	38,38^{a}	1,69	32,10^{a}	75,70	10,51	0,33
T2	1,088	40,33^{b}	1,55	33,55^{b}	76,64	10,48	0,11
T3	1,088	40,23^{b}	1,70	33,51^{b}	77,58	10,55	0,07
SEM	0,0005	0,047	0,063	0,245	0,593	0,582	0,062
P-level	0,630	0,011	0,574	0,022	0,775	0,892	0,172

They reported that phytase supplementation poultry diet improved protein digestion and utilization. Moreover, Ravindran *et al*. (1999) and Kies *et al*. (2001) reported that phytase supplementation in poultry diet increased nitrogen retention in broiler chickens. Similar results obtained by Klis *et al*. (1996) in layer hens. Moreover, exogenous phytase is effective in improving utilization of phytase to bound mineral in the diet (Biehl *et al*., 1995).

There is evidence to indicate that hydrolysis of phytate by microbial supplementation has improved absorption or retention of calcium, manganese, iron and magnesium in poultry (Qian *et al*., 1997; Sebastian *et al*., 1998; Ravindran *et al*., 2000).

Egg Quality

Data related to egg internal quality traits including egg weight, yolk and albumen weight, yolk and albumen percentage, yolk:albumen ratio, Haugh unit, albumen and yolk pH are presented in Table 3. The addition of phytase enzyme levels 250 and 500 FTU/kg of feed during the experimental period illustrated in data of Table 3 shows that main effect of phytase supplementation on egg weight was significant (P<0,01). Results showed that phytase supplementation improved egg weight, yolk and albumen weight.This results just as results of Peter (1992) indicated that laying hens fed a diet with phytase had significantly higher egg weight than hens fed on the same diet without supplemental phytase (control).

Also, Ciftci *et al*. (2005) who reported that the highest egg weight was determined in 600 U of microbial phytase kg^{-1} enzyme group (70,29g) and these following the 300 U of microbial phytase kg^{-1} enzyme group (67,37 g) and control group (64,31 g) respectively (P<0,01). While presented results are disagreement with findings of Scott *et al*. (1999) reported that, phytase supplementation had no significant effect on egg weight. The same was found by Yossef *et al*. (2001) who reported that phytase supplementation was not significant effect on egg weight at the average of 5- all nonphytate phosphorus levels. Egg weight, albumen and yolk weight were significantly increase by phytase supplementation (P<0,01). Keshevarz (2000) also reported that phytase supplementation significantly increased egg size laying hens. Phytase supplementation to the control diet 250 FTU/kg and 500 FTU/kg increased (P<0,01) albumen percentage from 58.40% to 59.49% and 58,86% respectively. But phytase supplementation was not increased yolk percentage and yolk: albumen ratio.

The highest yolk weight was determined in containing 500 FTU/kg enzyme group (17,21g) and those following control group (16,57g) and 250 FTU/kg enzyme group (16,45g) respectively (P<0,01). Haugh unit and yolk pH were not differed by dietary treatments (P>0,05). But albumen pH was significantly higher in 500 FTU/kg phytase supplemented group. In accordance with the present study, Gordon and Roland (1998) found that supplemental phytase significantly increased egg weights during weeks 4 and 6 (starting at 58 weeks of age) when diets contained 0,1% NPP. Peter (1992) noticed that layers fed diet with phytase had significantly higher egg weight than hens fed same diet except phytase. Several authors came up with the opinion that supplementation of phytase enzyme in poultry diet leads to improve in protein digestion and utilization (Kornegay, 1996; Sebastian *et al*., 1997) which might be the reason of deposition of augmented amount of egg content leading to bigger sized eggs. Mohammed *et al*. (2010) however, reported that phytase supplementation caused a significant increase in hen-day egg production but egg weight at the same time decreased significantly. Significantly (P<0,05) higher egg weight was obtained in hens of phytase supplemented groups compared to the control group likely because of an improvement in the digestion and utilization of protein by the hens resulting from phytase supplementation in the diet (Kornegay, 1996; Sebastian *et al*., 1997).

Phytase supplementation generally improved egg weight, yolk and albumen weight.

Shell Quality

The effect of treatments on some egg shell quality parameters are presented in Table 4. Egg shell strength, specific gravity of eggs, egg shape index and eggshell percentage were not significantly different among the treatments. However, egg shell weight and shell thickness were showed a significant (P<0,01) difference, that of phytase supplemented groups being the highest. As it was in this experiment, Panda *et al*. (2005) and Francesch and Brufau (2005) reported that specific gravity didn't influenced by phytase supplementation. Several researchers have demonstrated that phytase supplementation of 100 to 2 000 phytase units (FTU/kg) to diets containing approximately 0,10% dietary NPP has positive effects on egg production, egg mass, egg specific gravity, bone ash and egg shell quality by improving utilization of P (Van der Klis and Versteegh, 1996; Boling *et al*., 2000a; Boling *et al*., 2000b; Francesch *et al*., 2005). No effects on egg weight or egg specific gravity were observed by Carlos and Edwards (1998) when 600 FTU/kg was added to a 0,1% nonphytate phosphorus layer diet.

Um *et al*. (1999) observed no effect on eggshell strength, egg specific gravity, or eggshell thickness when 250 FTU/kg was added to diets with 0,26, 0,21 and 0,16% nonphytate phosphorus but did find these parameters to be lower when diets with 0,11% nonphytate phosphorus were used. Keshavarz (2000) also observed that the main effect of phytase on specific gravity was significant, as it was lower in the presence of phytase in the diet during the 30 to 42 and 54 to 66 week periods, respectively. In contrast, Gordon and Roland (1998) reported that phytase supplementation in diets with low NPP (0,1%) improved specific gravity. Lim *et al* (2003) found that supplementation of phytase in hens at 21 to 41 weeks of age did not affect specific gravity. Also, no phytase supplementation effect on specific gravity was observed by Boling *et al*. (2000) during the 20 to 70 weeks period, when maximum concentrations of NPP in diets were 0,2%. Broken and soft shell egg was not significantly affect by phytase supplementation. But there was a numerically decrease in the percentage of soft-shelled, broken, and cracked eggs with the addition of phytase to the diet in the current study. Lim *et al*. (2003) documented that phytase supplementation decreased the percentage of broken and soft shelled eggs. Studies have demonstrates that addition of phytase improves performance, and Ca and P digestibility, in layers fed on a maize and soybean-based diet (Lim *et al*., 2003; Panda *et al*., 2005; Wu *et al*., 2006). Similarly, Lim *et al*. (2003) found that supplementation of microbial phytase (300 U/kg) in the diet of laying hens can improve egg production and decrease the number of broken and soft eggs. The beneficial effects of phytase on egg shell quality were well documented by Lettner *et al* (1995), Kaminska *et al* (1996), Gordon and Roland (1998) and Rao *et al*. (1999). However, Panda *et al*. (2005), also obtained that addition of phytase to the 1,2 g/kg low phosphorus diet improved both shell weight and shell thickness.

Results showed that phytase supplementation significantly improved egg weight, albumen and yolk percentage, yolk and albumen weight, egg shell weight, egg shell strength and egg shell thickness. The present results are agreement with Punna and Roland (1999), Narahari and Jayaprasad (2001) and Metwally (2006) they found a beneficial effect of phytase supplementation on shell quality. Increasing shell thickness as a result of phytase supplementation due to. First the numerically improving shell breaking strength and second to beneficial effect of phytase supplementation on the utilization of phosphorus by poultry (Liebert *et al*., 2005; Pulmstead, 2007). Also, the present result are agreements with Jalal and Scheideler (2001) they indicated that there were not significant effects of phytase supplementation in normal, corn-soybean meal diets on dry and wet shell percentage. While the present results are disagreements with Casartelli *et al*. (2005) who studied that the effect of phytase supplementation (0, 1000 FTU kg^{-1}) on egg quality parameters from 32-48 weeks of age and from 48-64 weeks of age and found that, in the first trial, phytase improved shell percentage but, during the post peak period, there were no significant effect of phytase addition. From our results, we concluded that supplemental phytase has beneficial effects on the performance and egg quality of free-range laying hens. Phytase was added to layer diets, has positive effects on egg weight. On the other hand, There was no significant differences (P>0,05) on Haugh unit, egg specific gravity and shell breaking strength when laying hen fed with corn-soybean meal base diet supplemented with 250 or 500 U of phytase/kg diet.

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