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Effect of Vitamin D_3 and/or Zeolite Supplementation to Laying Hen Rations Added Microbial Phytase on Performance and Enterprise Income

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Abstract: The aim of this study was to examine the effect of vitamin D_3 and/or zeolite supplementation in presence of phytase enzyme on performance and enterprise income in laying hens. A total of 120, 28-weeks-old laying hens were used for the study. The laying hens were separated to 4 equal groups (5 replicates). The treatment groups were as follows: control diet (300 FTU phytase per kg), trial 1 diet (300 FTU phytase + 400 IU vitamin D_3), trial 2 diet (300 FTU phytase + 400 IU vitamin D_3 + 2% zeolite) and trial 3 diet (300 FTU phytase + 2% zeolite). The experimental period was 16 week. Body weight and feed conversion ratio were not statistically different between groups. Egg production was significantly lower in the phytase and vitamin D_3 added group than in the phytase added group on days 29-56. Feed intake was lower in the phytase added group than in the phytase and zeolite added group on days 85-112 ($p<0.05$). On days 1 and 112, egg weight was significantly lower in the phytase added group than in the other groups. The control group had higher income than the other groups.

Key words: Laying hens, phytase, vitamin D_3, zeolite, performance, economic analysis

INTRODUCTION

Feedstuffs used in poultry feeding are usually from vegetal sources and great portion of requirements for Calcium (Ca) and Phosphorus (P) are met by these feedstuffs. The 60-80% of P in plants are bound to phytic acid (phytate-P) which has low utilization ratio for animals (Carlos and Edwards, 1998). Low utilization of phytate-P is resulted in increase of the proportion of inorganic P supplementation to diet and the environmental pollution by excretion of P in feces (Angel *et al*., 2002).

Phytase enzyme releases P utilized easily in animals by hydrolizing phytate molecules (Carlos and Edwards, 1998). Denbow *et al*. (1998) reported that addition of phytase enzyme to diet decreased 50% of P excretion. Nelson *et al*. (1968) demonstrated that addition of phytase enzyme to feeds was an effective way to increase P availability to poultry. Lonnerdal *et al*. (1989) reported that dephytinization of soybean meal increases zinc (Zn) availability in chickens.

Performance of broilers may be increased in diets supplemented with phytase; phytase supplementation of broiler diets improved growth and bone mineralization and decreased mortality (Qian *et al*., 1996). Dietary P requirement can be decreased by 1 g kg^{-1} by phytase supplementation to diet (650-900 units g^{-1}) without influencing performance of broilers (Vogt, 1992).

Vitamin D_3 has a special role in hens due to its role in Ca and P homeostasis in the body and due to its influence on shell calcification and egg production (MacWhirter, 1994). Activity of phytase enzyme is related to Ca: P ratio and/or vitamin D_3 level of diet. Also, vitamin D_3 was directly related to Ca and P metabolism and increased phytate-P utilization (Qian *et al*., 1997). Qian *et al*. (1997) reported that the retaining of Ca was increased by addition of vitamin D_3 at the presence and absence of phytase enzyme in broilers fed corn-soybean basal diet.

The other feed additive is zeolite which is affect the utilization of dietary Ca and P. Zeolite has been used as adsorbent in animal nutrition due to its adsorptive ability on toxic agents such as aflatoxins (Ortatatli *et al*., 2005) and has been reported to increase the utilization of various nutrients (Willis *et al*., 1982). A synthetic zeolite, Sodium Aluminosilicate (SAS), had beneficial effects on the performance of poultry (Roland and Orban, 1989).

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As in all animal production activity, feed cost constitutes primary importance in poultry production (Aral *et al*., 1999). Therefore, technical achievement in new practices should be accompanied economic efficiency.

The objective of this study was to examine the effect of vitamin D_3 and/or zeolite supplementation in presence of phytase enzyme on laying rate, feed intake, feed conversion ratio, egg weight and on enterprise expenses and income in laying hens.

MATERIALS AND METHODS

A total of 120 28-week-old Lohman LSL layers were used for the study. The hens were reared in a completely enclosed house with ventilation fans. Lighting schedule followed a 16 h light: 8 h dark cycle. All hens were housed in individual cages. The laying hens were separated to 4 equal groups (5 replicates). They were fed a corn and soybean meal basal diet (NRC, 1994). The treatment groups were as follows: control diet (300 phytase units (FTU) phytase (from *Aspergillus niger*) (Natuphos 600, BASF Corp., Mt. Olive, NJ 07828 USA) per kilogram), trial 1 diet [300 FTU phytase + 400 IU vitamin D_3], trial 2 diet (300 FTU phytase + 400 IU vitamin D_3 + 2% zeolite (a natural zeolite, clinoptilolite) (Zeotech Corp., Albuquerque, NM 87107 USA)) and trial 3 diet (300 FTU phytase + 2% zeolite). The experimental period was 16 week. Feed and water were provided *ad libitum* during the experiment. Composition and calculated nutrients in diets are shown in Table 1.

Feed intake was determined by measuring the amount of daily given and monthly remained feed. Egg weights were determined by weighing solid and damaged eggs monthly.

Feed conversion ratio (kg feed/dozen eggs) was calculated for each group. Egg production was defined as number/day for each group during the experiment. For economic evaluation, partial budget analysis was used and the changes in expenditure and income were taken into consideration to compare groups (Aras, 1988). The feed costs of control and experimental groups and the sale price per egg were based on the marketing price at the end of the study.

Data were compared by using analysis of variance (ANOVA, Duncan's multiple-range test) between groups. Results are presented as mean±standard error. All statistical analysis was performed using software package program (SPSS for windows, Standard version 10.0, 1999, SPSS Inc., Headquarters, Chicago, IL, USA). A significance level of $p<0.05$ was employed in the analysis of data from groups (Snedecor and Cochran, 1980).

Table 1: Composition and calculated nutrients in diets (%)

Diets	Control (P)	Trial 1 (P+D_3)	Trial 2 (P+D_3+ZE)	Trial 3 (P+ZE)
Composition of nutrients				
Corn	63.00	63.00	63.00	63.00
Soybean meal, dehulled	24.00	24.00	24.00	24.00
Vegetable oil	1.20	1.20	1.20	1.20
Limestone	7.58	7.58	7.58	7.58
Dicalcium phosphate	1.06	1.06	1.06	1.06
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25
DL-Methionine	0.16	0.16	0.16	0.16
Iodized salt	0.50	0.50	0.50	0.50
Sand	2.00	2.00	-	-
Zeolite	-	-	2.00	2.00
Phytase (FTU)	300	300	300	300
Vitamin D_3 (IU)	-	400	400	-
Calculation of nutrients				
Crude protein	16.00	16.00	16.00	16.00
Metabolizable energy (kcal kg^{-1})	2750	2750	2750	2750
Calcium	3.50	3.50	3.50	3.50
Phosphorus, total	0.50	0.50	0.50	0.50

[1]Provided per kilogram of diet: vitamin A, 4,400 IU; vitamin D_3, 1,000 IU; vitamin E, 11 IU; riboflavin, 4.4 mg; d-pantothenic acid, 12 mg; nicotinic acid, 44 mg; choline chloride, 220 mg; vitamin B_{12}, 9 μg; vitamin B_6, 3 mg; menadione sodium bisulfite complex, 2.33 mg; folic acid, 3 mg; biotin, 0.3 mg; thiamin, 2.2 mg; ethoxyquin, 125 mg. [2]Provided per kg of diet: manganese, 75 mg; zinc, 75 mg; iron, 75 mg; copper, 5 mg; iodine, 0.75 mg; selenium, 0.1 mg

RESULTS

The effects of the different dietary treatments on the performance of laying hens are presented in Table 2. Body weight and feed conversion ratio were not statistically different between groups ($p>0.05$). Egg production was significantly lower in the phytase and vitamin D_3 added group than in the phytase added group on days 29-56. Feed intake was lower in the phytase added group than in the phytase and zeolite added group on days 85-112 ($p<0.05$). On days 1 and 112, egg weight was significantly lower in the phytase added group than in the other groups. However, it was significantly lower in the phytase added group than in the phytase and zeolite added group throughout the study (on days 1-112).

The economic comparisons of groups in laying hens fed different rations are shown in Table 3. The control group had higher income than the other groups. The cost of feed in the control group was lower than the other groups due to there were no any feed additives. In the other 3 groups, not only the cost of feed was higher but also egg production was lower or equal. Different feeding applications were not resulted in the significant increase of egg production income.

Table 2: Performance criteria in laying hens fed rations added microbial phytase and supplemented vitamin D_3 and/or zeolite (n = 30)

	Groups			
Days	Control (P)	Trial 1 (P+D_3)	Trial 2 (P+D_3+ZE)	Trial 3 (P+ZE)
Body weight (g)				
1	1,583.66±10.55	1,567.33±10.26	1,582.67±08.98	1,579.33±10.45
112	1,652.00±11.10	1,666.90±09.11	1,684.28±08.74	1,677.04±10.52
Feed intake (g/hen/d)				
1-28	118.85±0.05	118.94±0.03	118.95±0.06	118.88±0.05
29-56	118.35±0.31	117.72±0.28	118.10±0.10	118.03±0.23
57-84	117.26±0.19	118.43±0.23	118.03±0.23	118.41±0.28
85-112	117.11±0.28^b	117.72±0.33^{ab}	117.60±0.18^{ab}	118.18±0.19^a
1-112	117.89±0.32	118.20±0.16	118.17±0.13	118.37±0.12
Feed conversion ratio (kg feed/dozen eggs)				
1-28	1.57±0.05	1.71±0.02	1.62±0.05	1.52±0.02
29-56	1.45±0.02	1.52±0.01	1.46±0.01	1.46±0.01
57-84	1.43±0.01	1.44±0.01	1.45±0.02	1.45±0.01
85-112	1.43±0.01	1.44±0.01	1.43±0.01	1.44±0.02
1-112	1.47±0.02	1.53±0.03	1.49±0.02	1.47±0.01
Egg production (eggs/100 hens/days)				
1-28	91.19±1.73	84.53±1.35	88.69±1.80	93.93±1.02
29-56	97.85±0.45^a	93.07±1.76^b	96.81±0.35^{ab}	96.55±0.44^{ab}
57-84	98.21±0.27	98.20±0.62	97.74±0.85	97.94±0.68
85-112	98.21±0.50	98.50±0.63	98.78±0.55	98.42±0.64
1-112	96.37±0.94	93.57±1.77	95.50±1.15	96.71±0.52
Egg weight (g)				
1	56.96±9.26^b	58.30±0.30^a	59.00±0.25^a	58.98±0.47^a
28	60.06±0.31	60.64±0.34	60.64±0.36	60.64±0.34
56	62.20±0.28	61.76±0.27	61.60±0.27	61.90±0.25
84	63.08±0.22	62.62±0.30	62.84±0.29	62.40±0.27
112	64.52±0.31^c	65.44±0.29^b	65.84±0.32^b	66.96±0.28^a
1-112	61.36±0.21^b	61.75±0.20^{ab}	61.98±0.20^{ab}	62.10±0.22^a

^{a,b,c}different superscripts indicate significant differences between treatment groups ($p<0.05$). P: Phytase, D_3 vitamin D_3, ZE: Zeolite, Mean±Standart error

Table 3: Economic comparisons of groups in laying hens fed rations added microbial phytase and supplemented vitamin D_3 and/or zeolite (hen/112 days)

	Groups			
Cost and income	Control(P)	Trial 1(P+D_3)	Trial 2(P+D_3+ZE)	Trial 3(P+ZE)
Feed intake (g)	13,204	13,239	13,235	13,257
Feed cost (TL)	8.28	8.31	8.44	8.45
Egg production (number)	108	105	107	108
Income from egg (TL)	9.612	9.345	9.523	9.612
Income/Cost (TL)	1.33	1.03	1.08	1.16
Benefit/Cost (TL)	1.16	1.12	1.13	1.14

1 US Dollar: 1.5607 TL, 1 Euro: 1.9917 TL, TL: Turkish Liras, P: Phytase, D_3 vitamin D_3, ZE zeolite

DISCUSSION

Phytase supplementation (250 FTU kg^{-1}) to maize-soy layer diet eliminates inorganic P supplementation without affecting layer performance (Rao *et al*., 1999). Cromwell *et al*. (1995) reported that when phytase is added to a low P diet, gain is significantly improved in pigs. Similarly, addition of microbial phytase (750 FTU kg^{-1}) to a low P diet increased daily gain by 10.8% in growing swine (Li *et al*., 1998). Rats and mice also showed a positive response of live weight to exogenous phytase supplementation (Combs, 1998). Yu *et al*. (2004) showed that exogenous microbial phytase supplementation was effective in improving the growth performance of chickens fed low-nonphytate P (low-NPP) diet. Supplementation of basal diet (Ca 10 and NPP 3.0 g kg^{-1}) with phytase at 250 or 500 FTU kg^{-1} increased weight gains of chicks (Rao *et al*., 1999). Carlos and Edwards (1998) observed a positive effect of supplementing phytase on the body weight of laying hens. Likewise, broilers on diet supplemented with phytase at 49 days of age had significantly increased body weight compared to those on control diet (Huff *et al*., 1998). Keshavarz (2003) reported that hens fed diet without phytase supplementation lost more body weight than hens that received diet with 300 FTU. Contrarily, Peebles *et al*. (2007) noted that phytase supplemented diets reduced body weight in control layers. Ahmad *et al*. (2000) suggested that improvement in growth performance of chickens fed on microbial phytase supplemented diets may be attributed to the release of mineral from phytate mineral complex, the utilization of inositol by chicken, the increased starch digestibility and/or the increased utilization of protein.

Rao *et al.* (1999) reported that vitamin D_3 supplementation (200 mg kg^{-1}) to diet (Ca:NPP, 3.33:1) was ineffective in improving the weight gain of broilers. However, they also noted that when it was supplemented to another diet (Ca:NPP, 2.5:1), weight gain was improved, indicating that vitamin D_3 was not effective in improving the weight gain of broilers fed the diet having a wider Ca:P ratio. In broilers, body weight was observed to increase by addition of the microbial phytase and vitamin D_3 to their diets (Edwards, 1993). Conversely, Peebles *et al.* (2007, 2008) stated that supplementation of basal diets with phytase and vitamin D_3 caused a significant decrease in body weight of hens. Carlos and Edwards (1998) showed that addition of either 1,25-dihydroxycholecal-ciferol (1,25-$(OH)_2D_3$), phytase or their combination decreased the magnitude of weight loss as compared to hens fed basal diet. Li *et al.* (1998) indicated that when vitamin D_3 and phytase were added to diet, there was a numerical improvement in gain compared with diet containing only phytase ($p>0.05$). In agreement with findings by Li *et al.* (1998), the supplementation of diets with phytase or with phytase and vitamin D_3 did not cause a significant change in the body weight of birds in the current investigation (1652 vs 1666 g). Watkins and Southern (1992) indicated that when broiler chicks were fed sodium zeolite in low P diet, they experienced reduced growth rates. However, Dwyer *et al.* (1997) reported that clinoptilolite treated group in broiler chicks was not significantly different from controls for body weight (158 vs 159 g).

The results of Cromwell *et al.* (1995) study indicated that when phytase is added to a low P diet, feed intake is significantly improved in pigs. Improved feed intake by supplementing microbial phytase in broiler diet was also observed by Kornegay *et al.* (1996). In contrast, feed intake was not determined different between laying hens fed 0 or 300 FTU by Keshavarz (2003). Carlos and Edwards (1998) reported that there was also a slight insignificant increase in feed intake as phytase or 1,25-$(OH)_2D_3$ plus phytase were added. Similarly, Li *et al.* (1998) showed that there was not a significant difference between pigs fed only phytase and phytase plus vitamin D_3 combination for daily intake. These findings of Carlos and Edwards (1998) and Li *et al.* (1998) are consistent with the current study (117.89 vs 118.20 g). Moshtaghian *et al.* (1991) reported that supplementation of low P diet with SAS caused a marked reduction in feed intake ($p<0.05$) and SAS had not effective on feed intake for hens fed high P diet. Frost *et al.* (1992) stated that an effect was not seen as measured by feed intake because of feeding dietary sodium zeolite A. In the present study, the supplementation of zeolite to the feed with phytase increased feed intake at only days 85-112 ($p<0.05$, Table 2). This may be random since the means in two groups were very close (117.11 g in phytase group and 118.18 g in phytase plus zeolite group).

Huff *et al.* (1998) stated that there was not a significant effect of phytase supplemented feed (500 FTU kg^{-1}) on feed conversion ratio in broilers. Similarly, Keshavarz (2003) reported that feed efficiency was not statistically different between laying hens fed 0 and 300 FTU. Ahmad *et al.* (2000) indicated that they did not find a significant improvement in feed conversion ratio of broilers fed on maize and soybean diet supplemented with phytase. However, Simons *et al.* (1990) have reported improved feed: gain ratio till 2 weeks of age with supplemental phytase in broilers. Cromwell *et al.* (1995) also noted that when phytase (750 FTU kg^{-1}) is added to low P diet, feed efficiency is significantly improved in pigs (7.5%). Whereas, supplementation of low-NPP diet with phytase at 500 FTU kg^{-1} significantly decreased feed conversion ratio (1.716 vs 1.555) in broilers (Rao *et al.*, 1999). Same authors also reported that phytase supplementation (250 FTU kg^{-1}) significantly decreased feed conversion ratio (1.933 vs 1.476) in White Leghorn layers fed low-NPP diet. Li *et al.* (1998) stated that when vitamin D_3 and phytase were added to diet, there was a numerical improvement in feed efficiency compared with diet containing only phytase ($p>0.05$). The results of the present experiment are consistent with work of Li *et al.* (1998). Feed conversion ratio was not found significantly different between all groups in the study. Moshtaghian *et al.* (1991) reported that supplementation of low P diet with SAS caused a marked reduction in feed efficiency ($p<0.05$) and SAS had not effective on feed efficiency for hens fed high P diet. In the present study, probably since the diets contained normal P level, feed conversion ratio was not affected from zeolite supplementation.

Budor *et al.* (1995) showed increased egg production when microbial phytase was added to P-deficient diets. Improved egg production with phytase supplementation to low P diet (3.7 g kg^{-1}) was also reported by Gordon and Roland (1998). In contrast, Keshavarz (2003) reported that egg production was not statistically different between hens fed 0 and 300 FTU. Carlos and Edwards (1998) stated that addition of either 1,25-$(OH)_2D_3$ phytase, or their combination to diet did not have a significant effect on egg production during 8 weeks feeding period in laying hens 24 weeks of age. They also observed that addition of phytase or 1,25-$(OH)_2D_3$, or their combination to layer diets prevented rapid decrease in egg production because of *Mycoplasma gallisepticum* infection.

Similarly, Peebles *et al.* (2008) noted that dietary supplementation with phytase and 25-hydroxycholecalciferol (25-$(OH)_2D_3$) did not affect weekly or total egg production in layers. In the current study, egg production was higher in the phytase group than in the phytase plus vitamin D_3 group at days 1-56 of the experiment. After that, egg production in the phytase plus vitamin D_3 group increased. These results are parallel to the results of Carlos and Edwards (1998). These authors suggested that effects of phytase and 1,25-$(OH)_2D_3$ on egg production of layers differed with age and the added phytase and 1,25-$(OH)_2D_3$ acted together to prevent reduction in egg production in older hens. Although, Roland (1990) and Moshtaghian *et al.* (1991) reported that addition of SAS to P-deficient diet depressed egg production of laying hens ($p<0.05$), Frost *et al.* (1992) did not observe an effect on egg production because of feeding dietary sodium zeolite A. As similar to the report of Frost *et al.* (1992), a significant effect on egg production of zeolite supplementation to the groups was not observed in the present study.

Keshavarz (2003) stated that egg weight was not different between hens fed 0 and 300 FTU. Rao *et al.* (1999) reported that phytase supplementation to low-NPP diet significantly increased egg weight in layers, whereas vitamin D_3 supplementation significantly decreased egg weight. Carlos and Edwards (1998) observed that egg weight did not significantly change in groups supplemented 1,25-$(OH)_2D_3$, phytase or their combination of laying hens. Similarly Peebles *et al.* (2008) fed commercial layers with the unsupplemented basal diets or the diets supplemented with phytase and 25-$(OH)_2D_3$ and could not have found a significant effect on egg weight. Moshtaghian *et al.* (1991) stated that supplementation of low P diet with SAS caused a significant reduction in egg weight and SAS had no effect on egg weight for hens fed high P diet. Similarly, Frost *et al.* (1992) did not also determine an effect on egg weight due to feeding dietary sodium zeolite A in commercial Leghorns. In the current study, egg weights were lower in the phytase added group than in the other groups on days 1, 112 and 1-112. Since, this decrease in the phytase added group is also present in the beginning of the experiment, it is thought that statistical difference between the phytase group and the other groups is not important. In the experiment, it was not appeared the effect on egg weight of vitamin D_3 supplementation. May be, vitamin D_3 supplementation decreased egg weight in the phytase, vitamin D_3 and zeolite added group (trial 2) (Rao *et al.*, 1999) in comparison with the phytase and zeolite added group (trial 3) on day 112. In the trial 3, it seems to increase egg weight of zeolite supplementation.

CONCLUSION

The mean values belong to the all parameters were observed to be very close in all groups. Zeolite supplementation to the diet beside phytase supplementation improved feed intake and egg weight at the end of the study. Vitamin D_3 supplementation to the diet beside phytase supplementation did not indicate a significant effect on performance criteria due to probably adequate P level in the basal diet. Moreover, in the result of economic comparisons, when it was considered that the main goal of a management was maximum profit it was concluded that feeding with ration of control group was the most profitable application for layer hens.

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