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The Effect of Manganese and Phytase in the Diet for Laying Hens on Performance Traits and Eggshell Quality

Alp Onder Yildiz, Osman Olgun and Yusuf Cufadar
Department of Animal Science, Faculty of Agriculture, University of Selcuk,
42079, Kampus, Konya, Turkey

Abstract: An experiment was conducted to evaluate the effect of manganese and phytase in the diet for hens on performance and eggshell quality. The study was carried out on 108 H and N Brown Nick hens, from 22-42 weeks of age, allocated to nine experimental groups. Each treatment consisted of 4 replications (3 birds/ replicate). The birds were fed a basal diet containing 16% CP, 2800 kcal/ME/kg and 13.86 mg kg^{-1} Mn. Nine diets, arranged in a factorial design (3×3) with 3 levels of Mn (Manganese sulfate) diet (0, 35 and 70 mg kg^{-1}) and 3 levels of phytase preparation (0, 1000 and 5000 U) were used. The house had controlled ventilation and lighting (16 h day^{-1}). All hens were supplied with feed and water *ad-libitum*. The performance Body Weight Change (BWC) Egg Production (EP) Egg Weight (EW) Egg Mass (EM) Feed Intake (FI) Feed Conversion Ratio (FCR) and egg characteristics Egg Shape Index (ESI) Specific Gravity (SG) Albumen Index (AI) Egg Yolk Index (EYI) haugh unit, hu; Egg Shell Breaking Strength (ESBS) Egg Shell Thickness (EST) Egg Shell Weight (ESW) were not influenced by the dietary treatments during the experiment. It is concluded that supplementation manganese and phytase to diets containing a basal level of 13.86 mg kg^{-1} Mn is not necessary in laying hens at 22-42 weeks period.

Key words: Lying hens, manganese, phytase, performance, eggshell quality, traits

INTRODUCTION

Generally, corn and soybean meal are the major feedstuffs in the diets of poultry. Approximately two thirds of the P in the feedstuffs of poultry is phytic acid in the form of myo-inositol hexaphosphates. The availability of phytate phosphorus is very low in poultry due to the inability of the birds to produce sufficient amount of endogenous phytase (NRC, 1994). Phytic acid can form insoluble salts with Ca, Mg, Fe, Zn, Cu and Mn (Bedford and Schulze, 1998; Liu *et al*., 1998). When phytic acid is hydrolyzed by microbial phytase, it may release all phytate-bound minerals. Peter (1992) reported that laying hens fed a low nonphytate P diet with phytase had significantly higher egg production, egg weights and feed consumption than hens consuming the low nonphytate P diet without supplemental phytase.

A great deal of effort has been applied to improving eggshell quality in the fields of genetics, environmental condition and nutrition, especially mineral nutrition (Nys, 2001). In recent years, most of studies on nutrition effects on eggshell quality in laying hens have been focused on macrominerals (Ca, P) and vitamin D_3. Although, it is known, that enzymes related with some micro elements are important in mineralization process, the number of research on relationship between trace elements and eggshell quality is limited. Manganese (Mn), as cofactor of metaloenyzmes responsible for carbonate and mucopolysaccharides synthesis, play an important role in eggshell formation. Mabe *et al*. (2003) suggested that Zn, Mn and Cu could affect mechanical properties of eggshell by effect on calcite crystal formation and modifying crystallographic structure of eggshell. In previous study of Inal *et al*. (2001) found that 25 mg Mn kg^{-1} in the diets is sufficient for maximum egg production, egg weight and feed conversion, but for optimal eggs hell quality the requirement of layers is much higher.

In the study of Fassani *et al*. (2000), Mn addition (40-200 mg kg^{-1}) to diet for leghorn hens in the second cycle of production, improved shell thickness was observed when the diet was supplemented with 200 mg Mn kg^{-1}. Swiatkiewicz and Korelski (2008) reported that substitution of inorganic Zn and Mn (50 and 100 mg kg^{-1}) with amino acid complexes in the diet for laying hens had no effect on the laying performance (egg production, egg weight, egg mass, feed intake and feed conversion) but could alleviate the negative effect of hen age on eggshell breaking strength.

Corresponding Author: Alp Onder Yildiz, Department of Animal Science, Faculty of Agriculture, University of Selcuk, 42079, Kampus, Konya, Turkey

The aim of the present experiment was to study the effect of manganese and phytase in the diet for hens on laying performance and egg characteristics.

MATERIALS AND METHODS

At 22 weeks old 108 H and N Brown Nick laying hens were fed to 9 dietary treatments during the 22-42 weeks periods. The birds were randomly divided into 9 treatments. Each treatment consisted of 4 replications (3 birds/replicate). The birds were fed a basal diet containing 16% CP, 2800 kcal/ME/kg and 13.86 mg kg^{-1} Mn. Basal diets were shown in Table 1. Nine diets, arranged in a factorial design (3×3) with 3 levels of Mn (Manganese sulfate) diet (0, 35 and 70 mg kg^{-1}) and 3 levels of phytase (Rovaphos Phytase INTERCHEME, 500,000 FTU kg^{-1} phytase activity) preparation (0, 1000 and 5000 U) were used. For that calculated amounts of manganese sulfate and Rovaphos phytase were mixed with a small volume of the ration and then added to main ration. The house had controlled ventilation and lighting (16 h day^{-1}). All hens were supplied with feed and water *ad-libitum*.

Body Weight (BW) was obtained by weighting hens at the beginning and end of the experiment. Feed Intake (FI) and Egg Weight (EW) were recorded biweekly. Egg Production (EP) was recorded daily and Egg Specific Gravity (ESG) was measured monthly. Egg Mass (EM) was calculated from collecting data of EP and EW at biweekly via:

$$EM = \frac{EP \times EW}{Period} (days)$$

Feed Conversion Ratio (FCR; g of feed g^{-1} of egg) was calculated via:

$$FCR = \frac{FI\left(\frac{g\ of\ feed}{Hen/period}\right)}{EM\left(\frac{g\ of\ egg}{Hen/period}\right)}$$

Specific gravity and egg quality characteristics measurements were made on all collected eggs produced during 2 consecutive days of per at the end of 28 days period during the experiment. Specific gravity was determined same day using graded salt solutions ranging from 1.060-1.100 with gradations of 0.005. Egg shape index was determined by equipment (Digital calliper, Mitutoyo) that calculated the width: length ratio as a percentage. Egg yolk and albumen height was determined by digital height calliper.

Table 1: Composition of diet

Ingredients	%
Corn	53.700
Barley	10.000
Soybean meal (48% CP)[1]	18.300
Sunflower meal (31.20% CP)[1]	4.000
Vegetable oil (7800 kcal kg^{-1} ME)	3.300
Limestone	8.250
Dicalcium phosphate	1.650
Salt	0.350
Premix[2]	0.250
Lisin	0.020
Methionine	0.180
Total	100.000
Calculated nutrients	
Crude protein (%)	16.000
ME (kcal kg^{-1})	2799.000
Ca (%)	3.598
Available P (%)	0.419
Lisin (%)	0.757
Methionine (%)	0.407
Methionine + Cystine (%)	0.724

[1]Analyzed, [2]Premix provided/kg of diet; Zn 60000 mg, Fe 60000 mg, 5000 mg, Co 200 mg, I 1000 mg, Se 150 mg, vitamin A 4800000 mg, vitamin D_3 960000 mg, Vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B_1 1200 mg, vitamin B_2 2800 mg, niasin 10000 mg, Cal D-Pan 4000 mg, vitamin B_6 2000 mg, vitamin B_{12} 6 mg, folik acid 400 mg, D-Biyotin 20 mg, Kolin klorid 50000 mg

$$\text{Yolk index (\%)} = \frac{\text{Yolk height}}{\text{Yolk diameter}} \times 100$$

$$\text{Albumen index (\%)} = \frac{\text{Albumen height}}{\text{Albumen length and width}} \times 100$$

$$\text{Haugh unit} = 100 \times \log (AH + 7.57\text{-}1.7 \times EW^{0.37})$$

Where:
AH = Albumen Height
EW = Egg Weight

Egg quality analyses were completed within 24 h of the eggs being laid. Egg shell weight (%) was calculated by:

$$\frac{\text{Egg shell weight (g)}}{\text{Egg weight (g)}} \times 100$$

The eggs were subjected to determine characteristics of eggshell quality parameters (shell thickness and shell breaking strength) on all collected eggs produced during 2 consecutive days of per at the end of 28 days period during the experiment. Egg shell breaking strength was measured using a cantilever system by applying increased pressure to the broad pole of the shell using an instrument (Hisar terazi, istanbul). Shell thickness was measured at 3 locations on the egg (air cell and any side of equator) using a micrometer (Mitutoyo, 0.01 mm, Japan).

Data were subjected to ANOVA by using General Linear Model Procedure (GLM) in Minitab. Duncan's multiple range tests were applied to separate means. Statements of statistical significance are based on a probability of $p<0.05$.

RESULTS AND DISCUSSION

The data on performance Body Weight Change (BWC) Egg Production (EP) Egg Weight (EW) Egg Mass (EM) Feed Intake (FI) Feed Conversion Ratio (FCR) and egg characteristics Egg Shape Index (ESI) Specific Gravity (SG) Albumen Index (AI) Egg Yolk Index (EYI) Haugh Unit (HU) Egg Shell Breaking Strength (ESBS) Egg Shell Thickness (EST) Egg Shell Weight (ESW) were shown Table 2 and 3, respectively. The performance and egg characteristics were not influenced by the dietary treatments during the experiment. There were no found to be significant differences in all parameters of laying hens.

The results of the present study indicate that supplementation inorganic Mn and phytase of diet containing a basal level of 13.86 mg kg^{-1} Mn had no effect on performance and egg characteristics in laying hens. Similar results were observed by Zamani *et al.* (2005a, b), who found that supplementation Mn alone to diets had no any effect on FI and FCR. Also, same researchers stated that ESI, EST and EW were not influenced by addition Mn to diets of laying hens. In addition, adding 0.250 and 0.500 mg kg^{-1} inorganic and organic mineral mixture (Zn, Mn, Se) to diet of laying hens not influenced EP, FI, FCR, EM, EST, SG, HU (Fernandes *et al.*, 2008). However, Klecker *et al.* (2002) reported that substitution of 20 or 40% Mn and Zn from inorganic sources by their organic chelates significantly increased laying performance of hens from 20-60 weeks age. Swiatkiewicz and Korelski (2008) stated that substitution of inorganic Zn and Mn with amino acid complexes in the diet for laying hens had no effect on ESW, EST and SG, but improved ESBS in late phase of laying cycle. Similar results were found by Mabe *et al.* (2003) who reported no differences in ESW, ESI and SG between hens fed diet supplemented with inorganic and organic sources of Zn, Mn and Cu. Dale and Strong (1998) and Lim and Paik (2003) observed no beneficial effect of Zn and Mn organic sources on laying performance and eggshell quality measured as SG of eggs. In contrary Bunesova (1999) and Klecker *et al.* (2002) found positive effect of partial substitution of inorganic Zn and Mn sources with their organic forms on ESW and EST.

Panda *et al.* (2005) stated that addition of 500 FTU of microbial phytase/kg diet can reduce the NPP level to 1.2 g kg^{-1} in the layer diet, eliminate inorganic P supplementation and result in significant reduction of N and P excretion without affecting the performance of layers. Ciftci *et al.* (2005) reported that supplementation of microbial phytase (0, 300 and 600 U kg^{-1}) diet improved FI, EP, EW and FCR in layer hen. Lim *et al.* (2003) stated that supplementation microbial phytase at a level of 300 U kg^{-1} diet of laying hens can improve EP. Liu *et al.* (2007) reported that supplementation of phytase at 300 U kg^{-1} of feed in diets containing a high level restored the performance of layers. Um and Paik (1999) reported that supplementation of 500 U kg^{-1} diet influenced EP, EW, FI, SG and EST, but not influenced FCR, ESS and HU in laying hens. These differences among the literature may be due to from the use of phytase enzyme amount in diets.

Table 2: Effects of manganese and phytase supplementation to diet on performance

Effects	Body weight change (g hen^{-1})	Egg production (%)	Egg weight (g egg^{-1})	Egg mass (g/hen/day)	Feed intake (g/hen/day)	Feed conversion ratio (g feed g^{-1} egg)
Mn (ppm)						
0	203.6±39.50	91.26±1.25	60.99±0.60	55.78±0.96	108.6±1.57	1.95±0.040
35	190.0±23.80	92.59±2.01	59.81±0.58	55.42±1.20	106.6±1.77	1.93±0.029
70	241.8±31.70	91.05±1.80	60.06±0.78	54.42±1.47	106.3±2.53	1.96±0.033
Fitaz (U)						
0	205.6±28.90	93.29±1.37	61.23±0.48	56.75±0.94	107.2±1.41	1.89±0.024
1000	181.6±35.60	89.32±1.90	59.37±0.73	53.07±1.21	106.3±2.45	2.01±0.034
5000	248.3±30.90	92.29±1.64	60.26±0.68	55.79±2.71	108.1±2.02	1.94±0.035
Mn × Fitaz						
0×0	127.2±61.95	95.02±0.79	61.28±0.88	58.26±0.64	110.5±0.95	1.90±0.019
0×1000	158.0±70.60	88.40±2.68	60.37±1.39	53.41±2.09	107.2±2.57	2.02±0.075
0×5000	325.8±27.60	90.38±1.28	61.32±1.04	55.66±1.22	108.0±4.20	1.95±0.096
35×0	227.8±14.80	94.35±0.53	60.53±0.92	57.21±0.88	106.7±2.76	1.87±0.053
35×1000	170.7±30.80	89.89±4.79	59.88±1.44	53.80±2.76	105.9±3.84	1.98±0.055
35×5000	171.7±66.00	93.53±4.16	59.01±0.66	55.25±2.37	107.2±3.42	1.95±0.034
70×0	261.9±43.50	90.51±3.98	61.87±0.79	54.79±2.53	104.4±2.65	1.91±0.053
70×1000	216.0±85.90	89.66±3.03	57.85±0.78	52.01±1.89	105.6±6.63	2.03±0.057
70×5000	247.5±37.80	92.97±2.94	60.45±1.62	56.46±3.22	109.0±3.89	1.94±0.059

Table 3: Effects of manganese and phytase supplementation to diet on egg quality characteristics

Effects	Egg shape index (%)	Specific gravity	Albumen index (%)	Egg yolk index (%)	Haugh unit	Egg shell breaking strength (N)	Egg shell thickness (mm×10^{-2})	Egg shell weight (%)
Mn (ppm)								
0	77.92±0.37	1.084±0.0007	4.85±0.16	47.65±0.35	87.78±1.28	3.797±0.085	37.2±0.19	9.00±0.13
35	77.79±0.34	1.087±0.0012	5.18±0.09	47.05±0.41	90.03±0.68	4.111±0.109	37.4±0.21	9.38±0.15
70	78.29±0.53	1.085±0.0012	5.22±0.15	47.32±0.45	90.10±1.03	4.111±0.129	37.3±0.27	9.37±0.07
Fitaz (U)								
0	78.04±0.33	1.086±0.0008	5.29±0.13	47.71±0.37	90.99±0.85	4.199±0.090	37.3±0.17	9.34±0.09
1000	77.72±0.47	1.085±0.0011	4.98±0.16	47.72±0.45	88.29±1.21	3.876±0.133	37.1±0.26	9.25±0.15
5000	78.24±0.44	1.084±0.0008	4.98±0.13	46.59±0.31	88.63±0.95	3.944±0.103	37.6±0.22	9.15±0.14
Mn × Fitaz								
0×0	78.00±0.59	1.085±0.0013	5.18±0.17	48.59±0.13	90.06±1.17	3.959±0.163	37.4±0.29	9.24±0.18
0×1000	77.98±1.04	1.083±0.0008	4.73±0.41	47.75±0.52	87.12±3.36	3.746±0.159	36.8±0.31	8.96±0.17
0×5000	77.77±0.20	1.083±0.0013	4.63±0.18	46.61±0.63	86.17±1.60	3.686±0.115	37.5±0.32	8.81±0.30
35×0	78.51±0.45	1.087±0.0022	5.42±0.14	47.27±0.80	91.97±0.86	4.221±0.117	37.1±0.31	9.43±0.22
35×1000	76.98±0.67	1.088±0.0026	5.02±0.14	46.79±0.92	88.68±1.11	4.164±0.257	37.6±0.39	9.48±0.39
35×5000	77.88±0.45	1.085±0.0015	5.09±0.15	47.10±0.53	89.44±1.10	3.948±0.191	37.6±0.44	9.22±0.16
70×0	77.62±0.73	1.086±0.0008	5.26±0.35	47.29±0.69	90.94±2.27	4.417±0.114	37.4±0.33	9.35±0.05
70×1000	78.20±0.77	1.084±0.0011	5.18±0.25	48.61±0.73	89.06±1.74	3.719±0.249	36.8±0.60	9.32±0.15
70×5000	79.06±1.26	1.085±0.0008	5.22±0.27	46.05±0.41	90.29±1.72	4.198±0.154	37.6±0.47	9.44±0.18

The performance parameters were found to be similar during the experiment. Despite all the efforts for controlling the circumstances there are many factors such as hens age, use of enzyme and Mn source and dose, kind of nutrients with different sources and more importantly micro-flora kind and population content of the digestive system, in different regions seriously affect the performance parameters. The eggshell quality was not influenced by addition phytase and manganese in laying hens. The eggshell quality is one of the most important problems in poultry industry, influencing economic profitability of egg production and egg hatchability. High eggshell breaking strength and lack of shells defects are essential for protection against penetrating of pathogenic bacteria such as *Salmonella* sp. into the eggs (Swiatkiewicz and Koreleski, 2008). Trace minerals may affect eggshell quality due to their catalytic properties as constituents of key enzymes involved in the processes of membrane and eggshell synthesis or by direct interaction with calcium crystals during eggshell formation. Different results reported in literature on commercial products may be attributed to the specific chelating procedures employed, resulting in products with different bioavailabilities, stabilities and metabolization. Internal egg quality is usually evaluated by measurements of either white height or Haugh units, which is a function of the former conditions in supermarkets shelves. Although, egg storage is an essential trait for retailers, some changes in eff internal characteristics must be expected, e.g., water and CO_2 losses and pH increase (Decuypere *et al*., 2001).

CONCLUSION

The supplementation manganese and phytase to diets containing a basal level of 13.86 mg kg^{-1} Mn is not necessary in laying hens at 22-42 weeks period. More research is needed to determine the effects of manganese and phytase supplementation on performance and egg quality in laying hens.

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