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Full Length Research Paper

Effects of dietary humic substances on egg production and egg shell quality of hens after peak laying period

Ozturk Ergin*, Coskun Isa, Ocak Nuh and Erener Guray

Department of Animal Science, Faculty of Agriculture, University of Ondokuz Mayis, Samsun, Turkey.

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Ninety Isa Brown layers from 51 to 61 weeks of age were allocated to three treatment groups, namely H_0, H_1 and H_2. Control (H_0) hens were fed a commercial diet while H_1 and H_2 hens were fed a diet including 30 and 90 ppm dietary humic acid in liquid form, respectively. Hens were placed individually in cages in three tier batteries. Egg production (% hen-day) in the H_2 group was higher ($P < 0.05$) than control group. Egg mass of H_2 hens was higher ($P < 0.05$) than H_0 and H_1 hens. Total feed intake in H_2 group was significantly higher ($P < 0.05$) than H_1 group. Egg shell thickness of H_1 hens was higher ($P < 0.05$) than H_2 hens. Egg shell strength of H_1 hens was higher ($P < 0.05$) than control and H_2 hens. Egg weight, feed conversation ratio and yolk weight were not affected by dietary humic substances. These results indicated that the supplementation of 30 ppm humic acid into the diet may increase the egg shell strength without affecting egg production and feed efficiency compared to control counterparts.

Key words: Humic substances, poultry, feed additive, egg production, egg quality.

INTRODUCTION

Egg production and egg quality are the most important economic traits for layer farms. One of the most important factors affecting the profitability of egg production is the age-related decline in egg shell quality (Nys, 1999) due to reduction in mineral utilization and increase in egg shell surface as the hens aged. If eggs with poor shell quality pass through the system undetected, they can also constitute a risk to food safety. Therefore, control of the egg shell quality still has a part of play (Bain, 2005).

Since mineral premixes or feed additives can promote the utilization of minerals, they should be added to layer diets to improve egg shell quality. Humic substances (HS) that are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains (Klocking, 1994; MacCarthy, 2001) might be one of these feed additives for enhancing egg production and egg shell quality. However the knowledge on using humates as feed additives in animal nutrition is not conclusive and especially humates were used as a part of replacement therapy for digestive system disorders such as malnutrition and diarrhea (EMEA, 1999). Indeed, Yoruk et al. (2004) reported that supplementation of humate into the diet at level of 0.1 and 0.2% during the late laying period increased egg production, improved feed efficiency and reduced mortality. Moreover, it has been reported that addition of humate into layer diets at a level of 30 and 60 mg/kg (Kucukersan et al., 2005), up to 0.3 g/kg (Hayirli et al., 2005) or 2 g/kg (Kucukersan et al., 2004) can improve egg production, egg weight and feed efficiency. However, previous studies (Yoruk el al., 2004; Hayirli et al., 2005) showed that egg shell quality parameters were not affected by dietary inclusion of humate in layers.

These observations highlight that the importance of dietary HS supplementation may have critical consequence for egg production during layer period. On the other hand, due to the ability of humates to bind materials in certain environments and to release these materials under different environmental conditions (Shermer et al., 1998), dietary HS may prevent reduction in egg production and egg shell quality after peak laying period. Therefore, more information is still needed about the effect of HS on egg shell quality and egg production after

*Corresponding author. E-mail: eozturk@omu.edu.tr. Tel: 90-362-3121919 x 1144, Fax: 90-362-4576034.

peak period. The objective of the study was, therefore, to investigate effects of dietary HS on laying performance and some egg shell quality parameters after peak laying period.

MATERIALS AND METHODS

Ninety commercial layer hybrid (Isa Brown) hens aged 51 to 61 weeks were used in this research. These hens were allocated to three experimental groups (n = 30 for each group), namely H_0 (control), H_1 and H_2. While liquid HS did not added into H_0 diet, it added at the level of 10 (diet included 30 ppm humic acid and 2.5 ppm fulvic acid) and 30 ml/kg diet (diet included 90 ppm humic acid and 7.5 ppm fulvic acid) into H_1 and H_2 diets, respectively. The hens were placed individually in three-tier cage (50 X 46 X 46) system. Groups were composed of 10 replicates in each tear (30 hens for each of groups). Replicates were equally distributed into upper and lower level cages to minimize effect of cage level. The experiment was conducted in a deep pit house ventilated both naturally and mechanically, and illuminated both artificially and naturally through the windows and fed a commercial layer diets. Liquid HS and diets were purchased from a commercial company in Samsun (Table 1). Humic and fulvic acid contents of HS were determined in UV spectrophotometer (Grzybowski and Dudzinska, 2004) while all minerals of HS were determined by atomic absorption spectrophotometer. During the experimental period (June to August), hens were fed in mash form and watered *ad libitum* with 16 h continuous illumination per day by using the natural daylight. The experiments involving the laying period were conducted during Turkey's (41.2°N, Samsun) hot months (June to August). The highest and lowest mean monthly temperatures and relative humidity were 15.5 and 27.8°C (mean = 22.3°C), and 76.4 and 77.0%, respectively.

Hen-day egg production was recorded daily, while feed intake was measured weekly thoroughly the trial. Eggs weight and egg shell quality measurements were done on a biweekly basis on all the eggs laid on three consecutive days. The eggs were stored for 24 h at room temperature and then weighed. Feed conversation ratio (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced. An additional sample of 20 eggs for each treatment group was randomly collected every two weeks to assess egg quality parameters such as shell thickness, shell strength and yolk weight (Yoruk and Bolat, 2003).

Data were subjected to analysis of variance using one way ANOVA procedure of SPSS (release 10.5). Differences between means were ranked by Duncan's multiple range test of significance level of 5%.

Table 1. Composition of basal diet.

Ingredients ($g.kg^{-1}$)	Control (H_0)
Maize	428
Sunflower meal	150
Soybean meal	51.4
Wheat	149
Wheat bran	70
Meat and bone meal	63
Limestone	67.5
Acid oil	13
Salt	2.5
Vitamin-mineral premix†	2.5
Methionine	0.55
Lysine	2.56
Calculated content	
ME, kcal/kg dry matter ‡	2650
Crude protein (%)	16.00
Ca (%)	3.40
Available P (%)	0.47
Lysine (%)	0.70
Methionine (%)	0.37

†Each kilogram contained 15.000 IU vitamin A, 1.500 ICU cholecalciferol, 30 IU vitamin E (DL-ά-tocopheryl acetate), 5.0 mg menadione, 3.0 mg thiamine, 6.0 mg riboflavin, 20.0 mg niacin, 8.0 mg panthotenic acid, 5.0 mg pyridoxine, 1.0 mg folic acid, 15 μg vitamin B_{12}, 80.0 mg Mn, 60.0 mg Zn, 30.0 mg Fe, 5.0 mg Cu, 2.0 mg I and 0.15 mg Se.
‡Calculated from content of the feed ingredients.

RESULTS AND DISCUSSION

Chemical composition of humic substances and their amounts provided by humic substances into the experimental diets are presented in Table 2. No mortality was observed any of the experimental groups during the experiment. The egg production, egg weight, egg mass, egg yolk weight, feed intake, feed conversation ratio, shell thickness and shell strength values of treatment groups are shown in Table 3. Total egg production (number/70 days) and hen-day egg production (%) in H_2 group were higher (4.7%) than control group ($P < 0.05$). The egg production of H_1 group was higher than control, although the augmentation did not reach statistical significant. Therefore, H_2 group was higher in terms of egg mass (g/hen/day) than H_0 and H_1 groups ($P < 0.05$). Feed consumption of H_2 group was higher than those of H_1 group ($P < 0.05$). Egg shell thickness of H_1 group was higher (6.1%) than that of H_2 group. Shell strength value was higher (16.0 and 26.2%) in H_1 group than control and H_2 groups. There were no statistically significant differences among the control and other treatment groups in terms of FCR, egg weight and yolk weight.

Results of the present study indicated that the addition of 30 ppm dietary humic acid to layer diet after peak production may increase egg shell strength compared to control counterparts. In this study, the supplementation of the 90 ppm humic acid into diet increased the egg production compared to control and increased feed intake and decreased egg shell thickness and strength compared to the 30 ppm humic acid.

These results supported the idea that the effect of feed additives used to enhancing egg production, feed efficiency and egg shell quality are variable depending on their amount and origin, the technique of application and/or space and production stage of bird (Stackhouse and Benson 1989; Herzig et al. 1994; Trckova et al., 2005). In fact, in some studies egg production and feed

Table 2. Analysed chemical composition and amounts provided by humic substances into the experimental diets.

Contents	HS	Experimental groups		
		H_0	H_1	H_2
Dry matter (g/kg)	49.00	-	0.490	1.470
Humic acid (g/kg)	30.00	-	0.300	0.900
Fulvic acid (g/kg)	02.50	-	0.025	0.075
Crude protein (g/kg)	03.33	-	0.033	0.100
Minerals				
Ca (g/kg)	2.44	-	0.024	0.073
S (g/kg)	0.73	-	0.007	0.022
N (g/kg)	0.53	-	0.005	0.016
Mg (g/kg)	0.12	-	0.001	0.004
K (g/kg)	0.08	-	0.001	0.003
Fe (ppm)	92.74	-	0.927	2.782
NO^-_3 (ppm)	13.50	-	0.135	0.405
P (ppm)	3.57	-	0.036	0.107
Zn (ppm)	2.25	-	0.023	0.068
Cu (ppm)	1.10	-	0.011	0.033
Cr (ppm)	0.90	-	0.009	0.027
Ni (ppm)	0.55	-	0.006	0.017
Sn (ppm)	0.26	-	0.003	0.008
Pb (ppm)	0.03	-	0.000	0.001

Table 3. Effects of humic substances supplementation on egg production (total and hen-day), egg weight, egg yolk weight, egg mass, feed intake, feed conversation ratio, shell thickness and shell strength.

Parameters	Control (H_0)	H_1	H_2
Total egg production (no. for 70 days)	62.20 b	63.20 ab	65.10 a
Hen–day egg production (%)	88.80 b	90.00 ab	93.00 a
Egg weight (g)	57.38	57.30	58.69
Egg yolk weight (g)	16.80	16.80	17.40
Egg mass (g/bird/d)	51.21 a	51.90 a	54.63 b
Feed intake (g/bird/d)	117.2 ab	114.5 b	121.7 a
Feed conversation ratio (g feed/g egg)	2.33	2.27	2.24
Shell thickness (µ)	374 ab	384 a	362 b
Shell strength (kg/cm^2)	2.12 b	2.46 a	1.95 b

Means within a row with no common superscripts differ significantly ($P < 0.05$).
SEM: Standard error of the mean.

efficiency increased by supplementation of HS into layer diets (Yoruk et al., 2004; Hayirli et al., 2005), while these parameters were not affected (Yalcin et al. 2006).

Increase in the egg production and egg mass in the H_2 group compared to the H_0 group may be resulted from increase in feed intake of birds in the H_2 group. This situation may indicate that higher doses of HS are more effective than lower doses to increase feed intake. Our study was conducted during the hot season in the experimental region. However, the results with respect to feed intake show that heat stress has no deleterious effect on the feed intake. This is probably because the temperature during the experiment which was not in such a level that would cause a heat stress on birds. Therefore, decreased feed intake in the H_1 compared to H_2 group without improving feed efficiency and yolk weight was obtained which may be due to the detrimental effect of high HS on absorption of some nutrients and changes in metabolic profile. Also, some researchers (Klocking 1980; Stackhouse and Benson, 1989; Herzig et al., 1994) have reported that the effect of humic acids on heavy metal ion toxicity to experimental animals is dependant on the technique of application and the applied dose. However, neither increased mortality nor toxicity was observed in layers that were given high dose humic acid in this study.

Although there was a similar egg production between H_1 and H_2 groups, the fact that a lower feed intake in H_1 group compared to H_2 group may also confirm this thesis that the effect of H_2 on absorption of some nutrients and changes of metabolic profile was detrimental (Stackhouse and Benson 1989; Herzig et al., 1994). Indeed, Hayirli et al. (2005) noted that changes in metabolic profile due to humate supplementation may be related to alteration in partitioning of nutrient metabolism. Moreover, some trace elements in HS (Table 2) may act as co-factors, and consequently, increase the activity of several enzymes for digestion and utilization of nutrients (Hayirli et al., 2005). Such a beneficial effect of either low or high level of HS on the feed efficiency was not observed. Sterling et al. (2003) observed a positive correlation of feed intake with feed efficiency and egg mass. It may be understood that when feed intake was, therefore, evaluated together with egg production, egg weight and egg mass, feed efficiency did not differ among the groups.

An increase in egg shell thickness and strength by supplementation of 30 ppm humic acid compared to 90 ppm humic acid supported the idea that high level of humic acid in diet decreased Ca and P contents of blood compared to control or low level of humic acid (Rath et al., 2006). Moreover, the increase in egg shell quality by that of 30 ppm humic acid indicated that the low level of HS increased the cell wall permeability or absorption of nutrients. As previously known, increased permeability allowed easier transfer of minerals from the blood to the bone and cells (Enviromate, 2002). Decreased egg shell strength in the 90 ppm humic acid group compared to 30 ppm humic acid group may be attributed to enhanced egg mass and decrease in the egg shell thickness in this group (Lin et al., 2004). This situation may also be related to the antagonism between minerals or other nutrients in HS and in basal diet, although the antagonism and synergism were not determined in the present study.

By high level of HS, decreased absorption of Ca, Mg, Fe and P (Rath et al., 2006) (although it was not determined in the present study) may be due to a metal chelating effects of HS, which is effected by large number of carboxylic acid side chains (Klocking, 1994). Also, Grimes et al. (2004) reported that organic mineral complexes could increase availability compared with inorganic sources.

Reduced egg shell thickness and strength in the H_2 group can also be explained by the fact that the reduction in the absorption of P by high level of humic acid was twice more than that of Ca in the study of Rath et al. (2006), because the ratio of Ca to P are important factor as the primary determined for calcification of egg shell. It can be thought that the Ca and P levels of the basal diet could be changed by the contents of Ca and P of supplemental HS. The supplementations of HS into diets had rather little or no effect on the level of Ca and P in the basal diet (Table 2). It is not clear whether the HS effect on egg shell quality might be attributed to Ca metabolism, P utilization or egg shell mineralization. The exact impact of HS on egg shell quality remains to be elucidated. The increased egg shell quality in the H_1 group, despite the low feed intake may also be related to beneficial effect of low level of HS on absorption of nutrients such as Ca and P and/or changes in metabolic profile of these nutrients. The underlying mechanisms responsible for altered absorption of some nutrients and/or metabolic profile of these nutrients subsequent to the egg quality parameters described here remain unclear. However the observation of Rath et al. (2006) indicates that the nutritional properties of humic acid, stated above, particularly depend on its a supplementation level into broiler diet.The results of the present study indicate that egg shell quality measured by egg shell thickness and egg shell strength was more sensitive to supplemental HS than were egg performance criteria. Therefore, the low level of HS after peak laying period may improve egg shell quality and decrease feed intake although egg production and feed efficiency may not be affected.

Conclusions

These results showed that the supplementation of 30 ppm humic acid into layer diet after peak laying period can increase egg shell quality and decrease feed intake compared to 90 ppm of humic acid. Supplementation of 30 ppm humic also increased egg shell strength compared to the control counterparts without affecting egg production and feed efficiency. Because high HS supplementation after peak production period in layer hens have adverse effects on feed intake (although it increased egg production), feeding diet supplemented HS to layer hens during this period to improve egg shell quality should be treated with caution. Further studies of the underlying mechanisms of humic substance involved in egg production and egg shell quality after peak laying period is required.

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