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EFEFCT OF DIETARY SUPPLEMENTATION WITH HUMATE ON EGG PRODUCTION AND EGG SHELL QUALITY DURING THE LATE LAYING PERIOD OF LOCAL STRAIN HENS

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SUMMARY

This study was designed to investigate the effect of humate inclusion into diet of hens during the late laying period on egg production traits and egg quality. Two hundreds of El-Salam strain hens (n=200) 50 wk of age were allocated to four treatment groups, namely H_0, H_1, H_2 and H_3. Control (H_0) hens were fed a basal diet, while, H_1, H_2 and H_3 hens were fed the basal diet supplemented with 0.1, 0.25 and 0.4% humate, respectively. Active ingredients of humate were polymeric polyhydroxy acids (humic, fulvic, ulmic and humatomelanic acids). Egg production and egg weight were recorded daily and feed intake was evaluated biweekly. Also, a sample of 15 eggs from each group was collected randomly to determine egg quality every 28 days. Egg production and egg mass in the H_2 and H_3 were higher than H_0 and H_1 hens. Total feed intake in H_3 and H_2 groups were significantly higher ($P<0.05$) than H_1 hens. Egg weight, feed conversion ratio and egg quality (egg yolk%, egg albumen %, yolk index %, Haugh unit and egg shape index) were not affected by dietary humate. Egg shell quality values (shell %, shell thickness, cracked egg %, and shell ash %) of H_1 and H_2 hens were higher than H_3 hens. In conclusion, supplementation of humate at 0.25% during the late laying period could improve egg shell quality.

Keywords: Humate, feed additive, egg production, egg shell quality, Local chickens

INTRODUCTION

To enhance nutrient utilization, improve feed conversion efficiency, and maintain health status, inclusion of probiotics and humates in rations is preferable to antibiotics, primarily because they cause no harmful effects to consumers (Onifade *et al.*, 1999). Humates, a part of fertilizers, are derived from plant matter decomposed by bacteria (Senn and Kingman, 1973) and contain humus, humic acid, fulvic acid, ulmic acid and some microelements (Stevenson, 1994). Previous studies with respect to humates have focused mainly on the growth of germinal tissue in seed. The attitude of using humates as feed additives in animal nutrition is new. At first, humate was used as a part of replacement therapy for digestive system disturbances such as malnutrition and diarrhea and to increase feed efficiency in calves, dogs and cats (Kuhnert *et al.*, 1991).

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 of great consideration (Bain, 2005).

Since mineral premixes or feed additives can promote the utilization of minerals, they should be added to layers 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 and MacCarthy, 2001) might be one of these feed additives for enhancing egg production and egg shell quality. 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 *et al*., 2004 and Hayirli *et al.,* 2005) showed that egg shell quality parameters were not affected by dietary inclusion of humate layers .

These observations highlight that the importance of dietary humate supplementation may have critical consequence on egg production during laying period. On the other hand, due to the ability of humate to bind materials in certain environments and to release these materials under different environmental conditions (Shermer *et al.*, 1998), dietary humate may prevent reduction in egg production and egg shell quality after peak laying period. Therefore, more information is still needed about the effect of humate on egg shell quality and egg production after peak period. The objective of this study was to investigate effect of adding humate a laying diet on egg production and egg quality during the late laying period.

MATERIALS AND METHODS

This study was carried out at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

Hens and Management:

A total of 200 El-Salam strain 50- week old hens were used. They were randomly distributed into four groups each composed of 5 equal replicates and housed in 20 floor pens at 10 hens per pen under conventional conditions with access to feed and water *ad libitum* and photoperiod of 16 hours was maintained. Hens were fed on a layer diet (Table 1). This study lasted from 50^{th} to 62^{nd} weeks of age.

Treatments and Experimental protocol:

The experimental humate was supplemented to the basal diet of treatment groups at 0.0, 0.1, 0.25 and 0.40%. Biofarm® Dry, totally natural humate was purchased from Farmavet International Istnbul-Turkey. Each kg of humate contained 160 mg polymeric polyhydroxy acid (humic, fulvic, ulmic and humatomelanic acids), 663.3 SiO2 and other minerals (Mn, 50 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.5 mg and Al, Na, K, Mg and P in trace amounts).

Measurements:

Body weight at the beginning and the end of the experiment was individually recorded. Egg production, egg weight, cracked eggs and mortality were also recorded daily. Feed consumption was recorded weekly and feed efficiency was calculated. Egg quality measurements (egg shell, albumin and yolk weights) were measured using 15 eggs from each treatment group at the last 2 days of each month. Albumin, yolk and shell percentages, and Haugh units were also calculated. Yolk index percentage was calculated as yolk height divided by yolk diameter (Well, 1968). Exterior shell quality evaluation is based on shell thickness and shell ash. Shell ash was determined at 550^{o}C for 6 hours after drying at room temperature for 3 days. Shell thickness was measured with a micrometer gauge from three (top, middle and bottom) parts of the shell after shell membrane was separated from the egg shell.

Table 1. Composition* and calculated analysis of the basal diet

Ingredients	%
Yellow corn	64.0
Soybean meal (44%)	24.78
Wheat bran	1.00
Di-calcium phosphate	1.61
Limestone	7.91
DL-Methionine	0.10
Sodium chloride	0.30
Vit. & Min. Mixture**	0.30
Total	**100.00**
Calculated analysis:	
Metabolizable energy (kcal/kg)	2718
Crude protein %	16.02
Crude fiber %	3.46
Crude fat %	2.96
Calcium %	3.34
Available phosphorus %	0.42
Lysine %	0.89
Methionine %	0.39
Methionine + Cystine %	0.66

* As recommendation of Animal Production Research Institute, Agric. Res. Center

** Supplied per kg diet: Vit. A., 10000 IU; Vit. D3, 2000 IU; Vit. E 10 mg ; Vit. K3, 1 mg ; Vit. B1, 1 mg ; Vit. B2, 5 mg ; Vit. B6, 1.5 mg ; Vit. B12, 10 mg ; Niacin, 30 mg ; Pantothenic acid, 10 mg ; Folic acid, 1 mg ; Biotin, 50 mg ; Choline, 260 mg ; Copper, 4 mg ; Iron, 30 mg ; Manganese, 60 mg ; Zinc, 50 mg ; Iodine, 1.3 mg ; Selenium, 0.15 mg ; Cobalt, 0.1 mg.

All eggs in the experiment were visually checked for cracks and breakage under artificial lighting.

Statistical analysis:

Data were statistically analyzed using one way ANOVA (SAS, 1994). Before analysis, all percentages were subjected to logarithmic or arcsine values transformation (log10x+1) to approximate normal distribution. Significant differences among treatment means ($P \leq 0.05$) were separated by Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Results in Table 2 indicate that humate supplementation had insignificant effect on final live body weight. These results are in agreement with those reported by Kocabagli *et al.* (2002) and Karaoglu *et al.* (2004) who indicated that no significant effect on body weight and daily weight gain of broiler chickens fed diet with humate compared with the control group. While, Shermer *et al*. (1998) showed that the humic acid stabilizes the intestinal microflora and thus ensures an improved utilization of nutrients in animal feed, this leads to an increase in the live body weight of laying hens.

Table 2. Effect of humate supplementation on productive performance of El-Salam laying hens

Items	Control	Humate level, %			SEM*
		0.10	0.25	0.40	
Initial body weight , g	1700	1710	1705	1700	23.14
Final body weight, g	1750	1750	1767	1760	25.33
Egg laying rate (hen\day), %	45.24^{b}	46.43^{b}	47.62^{ab}	48.80^{a}	1.46
Egg weight, g	52.4	52.0	52.5	52.4	0.8
Egg mass, g/bird/d	23.70	24.14	25.00	25.59	0.62
Feed intake, g/bird/d	92.5^{b}	91.60^{b}	96.00^{ab}	100.0^{a}	1.51
Feed conversion, g feed: g egg	3.90	3.79	3.84	3.90	0.42
Mortality number	1	2	1	2	---

^{a,b,c}Mean in the same row with different letters are differ significantly at P≤0.05.
*SEM: Standard error of the mean.

The egg production, egg weight, egg mass, feed intake and feed conversion ratio values of treatment groups are shown in Table (2). Total egg production (laying percent,%) in H_2 and H_3 treatment groups were higher (by 5.3 and 7.8%) 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 and H_3 groups were higher in terms of egg mass than H_0 and H_1 groups (P<0.05). In Similar studies, Kucukersan *et al.* (2004) and Yoruk *et al.* (2004) found that supplementation of humate in layer diets at 0.1 and 0.2 % for 75 days during the late laying period caused egg production increase compared to control group. Contrary to this, Ceylan *et al.* (2003) found no effect of dry humate on egg production. Feed consumption of H_2 and H_3 groups were higher than those of H_1 group (P<0.05). This results agree with those of Ergin *et al.* (2009) who reported that feed intake was significantly higher at 90 ppm humate supplementation compared with 30 ppm humate for hens after peak laying period. Yoruk *et al.* (2004) found that humate with concentration of 0.1 and 0.2 % had no significant effect on feed intake in late stage of laying. However, Kucukersan *et al.* (2005) showed that the average daily feed consumption of hen fed diets with humic acid was significantly (P≤ 0.05) decreased compared with the control group.

There were no statistical significant differences among the control and other treatment groups in term of FCR. In a previous work by Ceylan *et al.* (2003) they did not find any effect of dry humate on FCR during the laying period. However, Kucukersan *et al.* (2004) and Yoruk *et al.* (2004) reported a significant improvement of FCR in the early laying period.

In this study, there was no effect of humate on egg weight (Table 2). These result agreed with the results of Ceylan *et al.* (2004) and Yoruk *et al.* (2004).

The increase in the egg production and egg mass in H_2 and H_3 treatment groups may be due to the increase in feed intake of birds compared with H_0 group.

These results may indicate that higher doses of humate are more effective than lower doses to increase feed intake. Therefore, decrease feed intake in H_1 group compared with H_2 and H_3 treatment groups without improving feed efficiency was obtained which may be due to the detrimental effect of high humate on absorption of some nutrients and changes in metabolic profile.

Although the egg production of H_1, H_2 and H_3 groups was similar , the fact that a lower feed intake in H_1 group compared with H_2 and H_3 treatment groups may confirm that the effect of H_2 and H_3 on absorption of some nutrients and changes of metabolic profile was detrimental Stackhouse and Benson (1989) and Herzig *et al.* 1994). 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 humate may act as co-factor and consequently, increase the activity of several enzymes for digestion and utilization of nutrients (Hayirili *et al.*, 2005). Such a beneficial effect of either low or high level of humate on the feed efficiency was not observed.

Streling *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.

Mortality for hens fed the control diet was not different from that for hens fed humate diets. There was also no effect of increasing the level of supplemental humate on mortality, (Table 2). Autopsy findings revealed that deaths were related to noninfectious causes. Little is known about the mechanism by which humate supplementation enhances the life span and improves production efficiency. However, available data consistently suggest that humate supplementation may benefit poultry production. Pukhova *et al*. (1987) reported that supplementation of Na humate in rats exposed to lethal doses of radioactivity increased the life span. In similar studies, it was shown that after high doses, supplemental humate alleviates toxicity of Cr in fish (Stackhouse and Benson, 1989) and Cd in chickens (Herzig *et al*., 1994) by reducing deposition of toxic metals in organs

Egg quality:

According to the results in Table 3, there were no effects of the experimental diets on shape index and yolk index. The results of Yoruk *et al.* (2004); Kucukersan *et al.* (2005) and Wang *et al.* (2007) support our findings regarding that there were no significant effects of humic acid supplementation on the studied egg quality traits. However,, egg shell ash significantly increased ($P<0.05$) in by H_1 and H_2 compared with H_0 and H_3 groups (Table 3). This result agree with that of Eren *et al.* (2000) who reported that shell ash increased by liquid humate addition to drinking water at different laying periods.

Cracked egg ratio was markedly decreased ($P<0.05$) by humate supplementation in H_1 and H_2 (Table 3) compared with H_3. Up to our knowledge, effect of humate on cracked egg ratio has not been investigated in any previous study. Only a related study by Gren *et al.* (2000) reported that dry humate supplementation to feed enhanced serum Na, K and tibia bone ash levels in broilers.

Table 3. Effect of humate supplementation on egg quality of Elsalam laying hens

Items	Control	Humate level, %			SEM*
		0.10	0.25	0.40	
Egg yolk, %	30.32	30.12	30.47	30.16	0.66
Egg albumin, %	56.25	55.26	55.24	57.24	1.02
Yolk index, %	48.60	48.00	47.60	48.00	0.19
Haugh units	78.75	78.54	79.25	78.52	2.62
Egg shape index	74.55	75.08	75.98	75.90	0.87
Egg shell, %	13.83^{b}	14.28^{a}	14.72^{a}	13.53^{b}	0.53
Shell thickness, mm	0.331^{b}	0.345^{ab}	0.356^{a}	0.321^{b}	0.007
Cracked egg, %	5.45^{a}	4.86^{b}	3.80^{c}	4.50^{b}	0.23
Egg shell ash, %	96.90^{b}	97.00^{a}	97.17^{a}	96.95^{b}	1.23

^{a,b,c}Mean in the same row with different letters are differ significantly at P≤0.05.
*SEM: Standard error of the mean.

An earlier report by Chen and Balnave (2001) suggested that carbonic anhydrase played an important role in egg shell formation and showed optimal activity in slightly alkaline medium. This study concluded that humate might have improved egg shell calcification by increasing Na and K levels, or causing other cation-anion changes. Although not well known yet, these types of metabolic events in the body may be one reason to positive effect on cracked egg ratio.

An increase in egg shell thickness by supplementation of 0.1 and 0.25% humate compared to 0.4% humate 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 at supplementation of 0.1 and 0.25% humate indicated that the low level of humate 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 thickness in the 0.4% humate group (H_3) compared to 0.1 or 0.25% humate treatment groups (H_1 and H_2) may be attributed to enhanced egg mass and decrease in the egg shell thickness in this groups (Lin *et al.*, 2004). This situation may also be related to the antagonism between minerals or other nutrients in humate and in basal diet, although the antagonism and synergism were not determined in the present study. However, the high level of humate decreases absorption of Ca, Mg, Fe and P. Rath *et al.* (2006) reported that this may be due to a metal chelating effect of humic acid which is affected 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 in the H_3 group can also be explained by the reduction in the absorption of P by high level of humic acid (Rath *et al.,* 2006) because the ratio of Ca to P are important factor as the primary determined for calcification of egg shell.

The improved egg shell quality in the H_1 and H_2 treatment groups, despite the low feed intake, may be related to beneficial effect of low level of humate 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 parameter described here remain unclear. However, the observation of of Rath *et al.* (2006) indicates that the nutritional properties of humic acid, stated above, particularly depend on it's a supplementation level into broiler diet.

The current study indicated that egg shell quality measured by shell ash, cracked egg ratio and egg shell thickness was more sensitive to supplemental humate than egg production or other egg quality criteria. Therefore, the low level of humate supplementation (0.25%) during late laying period improve egg shell quality but not egg production and feed efficiency.

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تأثير إضافة الهيومات فى العليقة على إنتاج البيض وجودة القشرة خلال فترة الإنتاج المتأخرة فى سلالة من الدجاج المحلى

رضا على حسن

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صُممت هذه الدراسة لدراسة تأثير أضافة الهيومات الى علائق الدجاج البياض خلال فترة الإنتاج المتأخرة على صفات انتاج البيض وجودة البيضة . تم أستخدام ٢٠٠ دجاجة من سلالة السلام المحلية عمر ٥٠ أسبوع وقسمت الى أربعة مجاميع وهى H_0 كنترول حيث تغذت على عليقة أساسية بينما المجاميع H_1&H_2&H_3 تغذت على علائق مضاف اليها ١٠. & ٠.٢٥& ٠.٤ % هيومات على التوالى . تم قياس انتاج البيض والعلف المستهلك يوميا وتم وزن البيض اسبوعيا وتم أخذ ١٥ بيضة من كل معاملة لتقدير صفات جودة البيضة مرة كل ٢٨ يوم . ومن أهم النتائج زادت نسبة وضع البيض فى المعاملة H_3 عن المعاملتين H_0&H_1 . فى حين أن كتلة البيض ووزن البيضة والكفاءة الغذائية وجودة البيضة الداخلية لم تتأثر بأضافة الهيومات. كما إن جودة القشرة فى المعاملة H_2 كانت أعلى عن المعاملة H_3 . نستخلص أن أضافة الهيومات (٠.٢٥%) خلال فترة الأنتاج المتأخرة يمكن تحسين جودة القشرة.