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Influence of humic acid addition to drinking water on laying performance and egg quality in Japanese quails

Oktay Kaplan*[1], Mehmet Avci[2], Nihat Denek[2], Murat Sedat Baran[1], Hüseyin Nursoy[3] and Faruk Bozkaya[4]

Dicle University, Faculty of Veterinary Medicine,
Department of Animal Nutrition, Campus of Dicle University, Diyarbakir, Turkey.
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ABSTRACT
The experiment was performed to investigate the effect of humic acid to drinking water on the laying performance and quality of eggs in Japanese quails. Hundred and forty, 9-weeks old female Japanese quails (Coturnix coturnix japanica) were randomly designated to four treatment groups forming 5 replicates each containing seven birds. Group I was the control group. Group II, III, IV were administered 250 ml/ton, 350 ml/ton, 450 ml/ton humic acid into drinking water, respectively. The egg characteristics were determined after 8th and 10th weeks of experiment. Addition of humic acid had a positive effect on the live weight change ($p < 0.05$) and yolk weight ($p < 0.01$). Egg shell thickness, albumen weight and yolk color were significantly ($p < 0.01$) lower in the experimental groups than that of control group. However humic acid had no effect on egg production, feed consumption, feed efficiency and other egg quality. In conclusion, data of our study demonstrated that liquid humate applied in drinking water has significantly improved live weight change and yolk weight. The positive effects of liquid humates, possibly related to improved nutrient utilization through various metabolic activities in the body are yet to be further investigated.

Key words: Egg quality, Humic acid, Quail.

INTRODUCTION
Japanese quail is an important species for its value of egg and meat production and as laboratory animal in bio medical research. Recently, many countries tended to prohibit antibiotics because of their side effects on birds and humans (Genedy and Zeweil 2003; Ibrahim *et al.*,2005). Antimicrobial feed additives are widely used in animal husbandry to improve animal production by increasing growth rate, enhancing feed conversion ratio and reducing disease risks (Arif *et al.*, 2015). However sub therapeutic use of antibiotics in poultry feeds has become undesirable because of the residuals in animal products, such as meat and eggs, and development of antibiotic resistant bacterial populations. Therefore in European Union, use of antibiotics as growth-promoting agents for poultry has been forbidden (Mutus *et al.*,2006; Guban *et al.*, 2006).

Humic acid (HA) naturally occurs by decomposition of organic matters like plants, and is a natural component of drinking water, soil, peat, sea sediments, brown or brown-black coals and lignite that are complex blends of poly aromatic and heterocyclic chemicals with poly carboxylic acid side chains (Rath *et al.*, 2006; Novak *et al.*, 2001)

HA forms a preservative film on the mucous epithelia of the gastro-intestinal tract against infections and toxins (Islam *et al.*, 2005). On the other hand HA increases the cell wall permeability allowing an easier transfer of nutrients from the blood to the cells. HA stabilizes the composition of intestinal flora and thus support the utilization of nutrients in animal feed (Islam *et al.*, 2005).

HA has anti-inflammatory and antibacterial effects in animals, and support immune system, therefore reduces mortality and increases growth in poultry (Islam *et al.*, 2005). HA shows a strong affinity to bind various substances, such as heavy metals (Madronova *et al.*, 2001), mutagens (Ipek *et al.*, 2008), minerals (Elfarissi and Pefferkorn, 2000), bacteria (Riede *et al.*, 1991),toxins (Sangeetha and Baskar, 2016) and aflatoxins (Arafat *et al.*, 2017).

The objectives of this study were to investigate the effects of HA addition to drinking water on egg production, feed consumption, water consumption, feed efficiency, egg weight and egg quality in Japanese quails.

MATERIALS AND METHODS
The experimental circumstances complied with animal welfare and suitable care of animals. A total of 140

*Corresponding author's e-mail: okaplan61@gmail.com
[1]Dicle University, Faculty of Veterinary Medicine, Dept. of Animal Nutrition and Nutritional Diseases, Diyarbakir, Turkey.
[2]Harran University, Faculty of Veterinary Medicine, Dept. of Animal Nutrition and Nutritional Diseases, Sanliurfa, Turkey.
[3]Bingöl University, Faculty of Veterinary Medicine, Dept. of Animal Nutrition and Nutritional Diseases, Bingöl, Turkey.
[4]Harran University, Faculty of Veterinary Medicine, Dept. of Animal Breeding, Sanliurfa, Turkey.

laying Japanese quail hens which had been in production for 9 weeks were, randomly, separated in a completely randomized design considering three treatments with five replicates each containing seven birds. The birds were housed in a 8 m x 7 m quail house equipped with four cages with wire mesh floor. Each cage compartment was equipped with a nipple drinker and a trough-type feeder. The poultry house was enlightened 16 h/day.

HA was not added to the drinking water of control group while 250 ml/ton, 350 ml/ton and 450 ml/ton of HA was added into the drinking water of Group II, Group III and Group IV, respectively. Chemical compositions of the diets were analyzed using the international procedures of AOAC (AOAC 2000). The formula for calculating ME has been described by Carpenter and Clegg 1956. Ingredients and chemical composition of the basal diet fed to Japanese quails are shown in Table 1.

The animals were fed in groups and the mean feed and water consumption of the groups was determined via weekly measurements. Feed efficiency was calculated using the following formula. Feed Efficiency= grams feed/grams egg mass (egg mass= (egg weight * % production)/100). Daily egg production in each group was recorded and eggs were weighed after storage at room temperature for 24 hours.

Fifteen eggs from each of the four treatment groups laid up to two successive days during the 8th and 10th weeks of experimental feeding were used for determining shape index, shell weight, yolk weight, albumen weight, shell thickness and yolk color. The egg yolk color was evaluated visually by means of the usual Roche scale. The egg shape index was measured by an electronic digital caliper. (Olgun and Yildiz 2014; Kumari *et al.*, 2016). The eggs were broken for measuring the weight of yolk and albumen. For weighing egg shells the shells were washed under slowly flowing water in order to remove albumen rests and dried in the open air for 24 hours. Then the shells were weighed along with the shell membrane. Average shell thickness was determined by measuring samples taken from sharp, bluntan dequatorial parts of the eggs. (Tyler. 1961; Card and Nesheim. 1972). Data were statistically analyzed by a one-way analysis of variance ANOVA, and the means were compared by the Duncan's multiple-range test (Steel and Torrie 1981).

RESULTS AND DISCUSSION

The results of egg production, feed consumption, feed efficiency, water consumption and egg quality are presented in Table 2. Live weight change ($p < 0.05$) and yolk weight were significantly ($p < 0.01$) higher in all the experimental groups than in the control group. Compared with the other groups, egg shell thickness and albumen weight were significantly higher ($p < 0.01$) in control group.

Yolk color was significantly ($p < 0.05$) lower in groups III, IV than that of other groups. No significant differences ($p > 0.05$) were found in egg production, feed consumption, feed efficiency water consumption and other egg quality between the control and treatment groups.

This study was designed to evaluate the effects of HA on egg production and some egg quality parameters in Japanese quails. The average live weight change was calculated as 15.88, 25.59, 28.40 and 20.11 g for the control group, Group II, III and IV respectively (Table 2). Mean live weight change of Group II was significantly higher than that of control group ($P < 0.05$). HA stabilizes the intestinal micro flora and thus ensures an advanced utilization of nutrients in animal feed. This leads to an increase in the live weight of laying hens (Shermer *et al.*, 1998) and broiler chicks (Arif *et al.*, 2015). Water and feed consumption of all groups were similar. Similar to the these findings several authors have reported that (Ceylan *et al.*, 2003; Yörük *et al.*, 2004) humate addition to diets and water has not affected feed intake

Feed efficiency (g feed/g egg) was calculated as 2.72, 2.81, 2.81 and 2.86 for the group I, II III and IV respectively (Table 2). Addition of HA to drinking water had no significant effect ($P < 0.05$) on feed efficiency. The results were similar to those reported by other researchers

Table 1: The composition of basal diet to feed animals (%).

Ingredients			
Corn	59.125	**Chemical analysis, drymatter (DM) basis**	
Soybean meal	28.00	Crude protein	19.98
Fish meal	4.00	Calcium	2.51
Vegetable oil	2.00	Total phosphorus	0.34
Dicalcium phosphate	0.90	**Calculated values**	
Calcium carbonate	5.40	ME (Kcal/Kg)	2900
Salt	0.30	Lysine	1.11
Vitamin mineral premix[a]	0.25	Methionine+cystine	0.70
DL-Methionin	0.025		
Total	100		

[a]**Vitamin premix provided the following per kg diet**: Vitamin A, 12500 IU; Vitamin D3, 1500 IU; Vitamin E, 31.25 mg; Vitamin K3, 3.75 mg; Vitamin B1, 2.5 mg; Vitamin B2, 7.5 mg; Niacin 25 mg; Cal. D-pantothenate 10 mg; Vitamin B6, 5mg; Vitamin B12, 0.019 mg; Folic acid 1 mg; Choline chloride 250 mg; Mn 100 mg; Fe 75 mg; Zn 75 mg; Cu 6.25 mg; Co 0.25 mg; I, 1.25 mg;Se 0.19mg.

Table 2: Effects of humic acid on laying performance and egg quality in quails (Mean±Standart Error).

Control	**Group I**	**Group II**	**Group III**	**P**	
Initial body weight(g/bird)	209.07 ± 2,34	210.89 ± 1.91	205.52 ± 2.38	210.38 ± 3.20	NS
Final body weight(g/bird)	224.95 ± 2.13	236.48 ± 3.94	233.92 ± 4.36	230.49 ± 2.74	NS
Live weight change (g/bird)	15.88 ± 0.71^{b}	25.59 ± 4.10^{ab}	28.40 ± 2.74^{a}	20.11 ± 4.56^{ab}	*
Water consumption (ml/bird/d)	42.08 ± 1.13	44.02 ± 1.29	44.45 ± 1.42	42.75 ± 1.79	NS
Feed consumption (g/bird/d)	28.81 ± 0.76	30.14 ± 0.40	28.65 ± 0.56	30.35 ± 0.57	NS
Egg production/hen/d (%)	90.98 ± 0.55	90.97 ± 0.53	88.31 ± 2.46	88.73 ± 1.82	NS
Feed efficiency (g feed/g egg)	2.72 ± 0.08	2.81 ± 0.04	2.81 ± 0.07	2.86 ± 0.06	NS
Egg weight (g)	11.93 ± 0.14	12.25 ± 0.18	11.93 ± 0.15	12.19 ± 013	NS
Yolk+Albumen weight	10.98 ± 0.14	11.31 ± 0.17	10.97 ± 0.14	11.20 ± 0.13	NS
Yolk+Albumen (% of egg weight)	92.01 ± 0.23	92.26 ± 0.22	91.98 ± 0.22	91.90 ± 0.19	NS
Egg shell weight (g)	0.95 ± 0.02	0.95 ± 0.03	0.96 ± 0.03	0.99 ± 0.02	NS
Egg shell ratio (% of egg weight)	7.99 ± 0.23	7.74 ± 0.22	8.02 ± 0.22	8.10 ± 0.19	NS
Yolk weight(g)	3.87 ± 0.11^{b}	4.06 ± 0.08^{a}	4.02 ± 0.06^{a}	4.10 ± 0.05^{a}	**
Yolk ratio (% of egg weight)	32.37 ± 0.72^{b}	33.12 ± 0.36^{a}	33.71 ± 0.42^{a}	33.61 ± 0.32^{a}	**
Albumen weight (g)	7.11 ± 0.11	7.25 ± 0.12	6.96 ± 0.12	7.11 ± 0.10	NS
Albumen ratio (% of egg weight)	59.64 ± 0.72^{a}	59.14 ± 0.43^{ab}	58.26 ± 0.47^{b}	58.29 ± 0.35^{b}	**
Yolk/albumen (rate %)	55.05 ± 2.31^{b}	56.19 ± 1.01^{a}	58.11 ± 1.18^{a}	57.80 ± 0.85^{a}	**
Egg shell thickness (mm)	0.206 ± 0.003^{a}	0.185 ± 0.003^{b}	0.185 ± 0.004^{b}	0.184 ± 0.004^{b}	**
Shape index	79.15 ± 0.441	78.18 ± 0.512	77.66 ± 0.698	78.38 ± 0.539	NS
Yolk color	6.10 ± 0.175ab	6.33 ± 0.182a	5.86 ± 0.133bc	5.50 ± 0.125c	**
Specific gravity	1.0755 ± 0.001	1.0762 ± 0.001	1.0768 ± 0.001	1.0722 ± 0.001	NS

a-c: Values bearing different alphabets in the same line indicates significant difference.
NS P > 0.05; v*** P < 0.001

(Küçükersan *et al.,* 2005; Eren *et al.,* 2000; Kocabagli *et al.,* 2002).

Differences observed between the groups for mean egg production were not statistically significant (Table 2). These findings are in accordance with those reported by Küçükersan *et al.*(2005) and Ceylan *et al.*(2003). Eggs from the control and treatment groups weighed as 11.93-12.25 g, which is coherent with literature rate of 10.39-10.79 g for quails (Baumgartner *et al.*, 2007). No significant difference in egg weight between the groups was observed. This result was in accordance with the results of Küçükersan *et al.* (2005);Ceylan *et al.* (2003) and Yörük *et al.*(2004) who reported that HA addition did not change the weight of eggs.

Addition of HA to drinking water decreased egg shell thickness in Japanese quails in the present study. Reduced egg shell thickness in the treatment groups can be explained by the decrease in the absorption of Ca and P by elevated level of HA probably due to chelating effect of HA for Ca and P (Rath *et al.*,2006). It can be thought that the Ca and P levels of the basal diet could be altered with the ingredient of Ca and P of supplemental HA. However in different studies (Ceylan *et al.*, 2003 and Yörük *et al.*, 2004) dry humate supplementation did not affect either shell strength or thickness.

The yolk weight of group III (4.10 g) was significantly higher than that in the control (3.87 g) (P < 0.001) while egg yolk percentage was not affected by HA addition. The results were in accordance with those reported by Yalcin *et al.*(2006).

Egg shell weight and shape index, defining was not significantly affected by addition of HA to drinking water in Japanese quails used in the present study (Table 2).This observation was similar to the findings of Küçükersan *et al.* (2005) ;Ceylan *et al.*(2003) and Yörük *et al.*(2004).

CONCLUSION

In conclusion, data of our study demonstrated that liquid humate administered in drinking water has significantly improved live weight change and yolk weight. The positive effects of liquid humates, possibly related to improved nutrient utilization through various metabolic activities in the body are yet to be further investigated.

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