Effects of Dietary Supplementation of Humic Substances on External and Internal Egg Quality of Oravka Hens

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Abstract
The aim of this study was to appraise the effect of humic substances (HS) in different concentrations on external and internal egg quality of laying hens. In total, 20 Oravka hens were allocated to 4 treatments, each containing 5 birds. The control birds were fed a diet without additives (0.00% HS), other treatment birds were fed with diets containing HS at 0.50%, 0.75 and 1.00%. The birds in all groups had available drinking water and feed mixtures ad libitum. The experimental period lasted 16 weeks. Also, 20 egg samples from each group were randomly every 2 weeks to assess egg quality parameters. Adding HS in different concentrations significantly increased ($P<0.05$) egg weight, albumen index, Haugh unit and yolk index and decreased yolk percentage ($P<0.05$), compared with control. There were no differences ($P>0.05$) in egg shape index, egg volume, eggshell weight, eggshell percentage, eggshell strength, eggshell thickness, albumen weight, albumen percentage, yolk weight and yolk colour among treatments.

Key words: egg, external quality, humic substances, internal quality, laying hen.

1. Introduction

During the past several years, probiotics, prebiotics, organic acids, phytogenics, zeolites, humates, and enzymes have all been used as feed additives for farm animals as alternatives to antibiotics due to their beneficial effect on health and growth performance combined with their lack of harmful effects on the consumer's health [1-3].
Humic substances (HS) are the final and stable biotransformation products resulting from decomposition of organic matter [4].

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The active components of HS consist of humic acid, humus, ulmic acid, fulvic acid, humin and some microelements. HS are successfully used in poultry nutrition in different forms and concentrations [5,6].
Humic substances commonly present in nature as they are created from the organic matter decomposition, and are normally found in the soil and natural water. Active components of HS consist of humic acid (HA), humus, ulmic acid, fulvic acid, humin and certain microelements. Humic acid is widely used as an alternative growth promoter for antibiotics in improving poultry performance and health. Moreover, supplementation of a commercial substance as a

source of HS through the drinking water or diet improved the feed consumption, feed efficiency and weight gain of broiler chickens, and also improved egg weight, egg mass, and egg production of laying hens [7].
Our study was focused on the effect of supplementation of different concentrations of HS on the external and internal quality of Oravka chicken eggs.

2. Materials and methods

2.1 Animal management and treatments

A total of 20 hens of the Oravka breed were allocated to its own covered shelters with straw litter and with access to a grass paddock; feeders and drinkers were available both outdoors and indoors.
The environmental temperature varied from 15 to 25 °C and the relative humidity from 65 to 75%. Artificial lighting was carried out in a pattern of 18 hours of light alternating with 6 hours of darkness. The ventilation in the house was natural with additional fans in order to ensure a minimum air exchange per hour.
The Oravka hens were divided to 4 groups (n=5): control birds were fed a diet without additives (0.00% HS), other treatment birds were fed with diets containing HS at 0.50, 0.75 and 1.00% in a powder form during laying period.
The Oravka hens were fed commercial feed mixture (Tekro Ltd., Nitra, Slovak Republic). Nutritional value of diet is shown in Table 1. The hens in all treatments had available drinking water and feed mixtures ad libitum.
The poultry in the experimental treatments received HS (Humac Ltd., Košice, Slovak Republic) with an 85% dry matter content; there was a minimum of 62% humic acid in the dry matter, a minimum of 48% free humic acids in the dry matter, a minimum of 9% fulvonic acids in the dry matter and a minimum of 9% minerals in the dry matter.

2.2 Egg quality parameters and analytical procedures

Chicken eggs were from Oravka breed in peak of laying period in sample size of 30 eggs from each treatment. The collected eggs were kept under room temperature, and egg quality was determined within 24 hours of collection.
The individual egg weight was measured using a laboratory scale Owa Labor (VEB Wägetechnik Rapido, Germany). The egg length and width were measured using digital calliper (Insize Ltd., China) with an accuracy of 0.01 mm.
Based on the measurements of egg length (L) and width (W) were calculated: egg shape index (SI) was calculated from the formula $SI = (W/L)*100$ [8] and volume of eggs $(V) = (\pi/6)*L*W^2$ [9].
After the eggs were broken, were cleaned to remove any albumen fragments and then naturally dried for 48 hours. Following this procedure, eggshell weight (with membrane) was measured using a laboratory scale Owa Labor (VEB Wägetechnik Rapido, Germany) and the percentage proportion of the eggshell in the egg was determined. Each calcified eggshell was roughly marked in 3 parts with equal length along the longitudinal axe, which is blunt end, equator zone and sharp end. The thickness of each eggshell piece was measured with a digital micrometre. The eggshell strength was determined manually using an Egg Crusher device (VEIT Electronics, Czech Republic).
The albumen weight was calculated as the difference among the egg weight and the yolk and eggshell weights. Albumen index (%) was determined by Alkan et al. (2010) [10] as the ratio of the thick albumen height (mm) to the average of width (mm) and length (mm) of this albumen measurement taken with a micrometre with 0.01mm accuracy. Haugh unit was calculated according to the procedure of Haugh as $\log(\text{height albumen} - 1.70 \times \text{egg weight}^{0.37} + 7.60)*100$ (1937) [11].
Yolk weight with 0.01 g accuracy was determined using a laboratory scale a laboratory scale Owa Labor (VEB Wägetechnik Rapido, Germany) and its percentage proportion was calculated. Yolk index (%) was measured as ratio of the yolk height (mm) to the yolk width (mm) by Funk (1948) [12] using micrometre with 0.01mm accuracy. Yolk colour was determined with La Roche scale (Hoffman–La Roche, Switzerland).
The proportion of eggshell, yolk and albumen relative to egg weight was expressed as eggshell or albumen or yolk weight/egg weight*100 [13].

2.3 Statistical analysis

The differences among the treatments were analysed with a one-way analysis of variance (ANOVA) by using the statistical program JASP 0.8.6 (2018) [14]. The results were evaluated using Duncan's multiple range test [15].

3. Results and discussion

3.1 Egg quality parameters

It was observed that there were significant ($P<0.05$) differences among treatments with respect to egg weight (Table 2). Our findings revealed a significant improvement in egg weight and egg mass in response to dietary HS, and this is in line with previous reports that HS exerted a beneficial effect on egg weight [16-21]. Our results are not consistent with Yorük et al. (2004) [22] who indicated that the addition of HS to layer diet has no positive effect on egg weight.

Egg shape index was not affected ($P>0.05$) by addition of HS in different doses to layer hen diet and we can characterize eggs in all treatments as normal eggs. Our results are in agreement with those reported by Yorük et al. (2004) [22] and Samya (2016) [21], who found insignificant effects of HS on egg shape index.

According to this the eggs were classified with respect to shape index (SI), namely as a sharp egg (SI<72), a normal (standard) egg (SI=72–76) or a round egg (SI>76) [8, 23].

3.2 Eggshell quality parameters

Eggshell thickness was not improved ($P\geq0.05$) by dietary HS. Eggshell strength was not improved ($P>0.05$) by dietary treatments. Eggshell strength did not vary ($P>0.05$) among the HS groups.

Results of the present study indicated that the addition of HS to layer diet no affected eggshell weight compared to control counterparts. In this study, the supplementation of HS into diet no affected eggshell percentage compared to the HS.

This no corroborates the previous findings of Ozturk et al. (2012) [24] that supplementation of HS in laying hens improved eggshell thickness. Also, HS exerted a beneficial effect on eggshell thickness and eggshell strength [19,25,26]. Conversely, Yörük et al. (2004) [1], Macit et al. (2009) [3], Yalcin et al. (2006) [27] and Hakan et al. (2012) [28] demonstrated that HS had no influence on eggshell thickness and strength.

Table 1. Nutritional value of complete feed mixture

Nutrient Ingredients	Unit	Feed mixture
Wheat	%	15.00
Maize	%	32.00
Soybean meal	%	19.20
Fish meal	%	3.00
Malt flower	%	3.00
Rapeseed meal	%	7.00
Sunflower meal	%	4.50
Monocalcium phosphate	%	1.00
Fodder salt	%	0.30
Animal fat	%	4.00
Calcium carbonate	%	10.00
Premix of additives[1]	%	1.00
Nutrient	**Unit**	**Feed mixture**
Crude protein	g/kg	200.00
ME_N	MJ/kg	11.70
Ash matter	g/kg	160.00
Fibre	g/kg	60.00
Lysine	g/kg	11.00
Methionine and cistine	g/kg	7.90
Ca	g/kg	35.00
P	g/kg	5.00
Na	g/kg	1.50

Notes: CP=crude protein; ME_N=nitrogen-corrected metabolizable energy; Ca=calcium; P=phosphorus; Na=natrium; MJ=megajoule; [1]active substances per kilogram of premix: vitamin A 15 000 IU; vitamin E 20 mg; vitamin D_3 2 000 IU; vitamin B_2 6 mg; vitamin B_{12} 20 μg; Mn 60 mg; Zn 40 mg; Fe 40 mg; Cu 6 mg; Se 0.2 mg.

Table 2. Effect of different HS concentrations on egg parameters

Parameter	0.00% HS	0.50% HS	0.75% HS	1.00% HS
Egg weight (g)	58.74±3.81[b]	60.12±3.92[a]	60.48±3.94[a]	60.81±3.99[a]
Egg shape index (%)	74.68±1.09	74.73±1.11	74.83±1.11	74.77±1.10
Egg volume (cm^3)	52.20±3.18	52.21±3.22	52.24±3.24	52.23±3.23

Values shown are mean ± SD (standard deviation)
[a,b] means in a row with different superscript differ significantly (P<0.05)

Table 3. Effect of different HS concentrations on eggshell parameters

Parameter	0.00% HS	0.50% HS	0.75% HS	1.00% HS
Eggshell weight (g)	5.76±0.28	5.97±0.31	5.99±0.34	6.03±0.35
Eggshell percentage (%)	9.81±0.41	9.94±0.42	9.91±0.41	9.92±0.42
Eggshell strength ($N.m^{-2}$)	30.76±4.97	30.97±5.02	30.86±5.05	30.74±4.99
Eggshell thickness (μm)	434.38±21.75	435.78±22.13	435.91±22.23	435.96±22.38

Values shown are mean ± SD (standard deviation)

Table 4. Effect of different HS concentrations on albumen parameters

Parameter	0.00% HS	0.50% HS	0.75% HS	1.00% HS
Albumen weight (g)	35.20±3.19	36.19±3.28	36.43±3.31	36.61±3.33
Albumen percentage (%)	59.92±2.67	60.20±2.78	60.23±2.81	60.21±2.77
Albumen index (%)	70.48±2.14[b]	71.87±2.22[a]	71.92±2.24[a]	71.88±2.24[a]
Haugh unit	79.56±1.99[b]	81.74±2.35[a]	81.96±2.41[a]	81.83±2.40[a]

Values shown are mean ± SD (standard deviation)
[a,b] means in a row with different superscript differ significantly (P<0.05)

Table 5. Effect of different HS concentrations on yolk parameters

Parameter	0.00% HS	0.50% HS	0.75% HS	1.00% HS
Yolk weight (g)	17.78±1.37	17.95±1.43	18.06±1.42	18.16±1.45
Yolk percentage (%)	30.27±0.78[a]	29.86±0.71[b]	29.86±0.73[b]	29.87±0.75[b]
Yolk index (%)	43.27±0.89[b]	45.03±0,91[a]	44.92±0.90[a]	44.95±0.90[a]
Yolk colour (°HLR)	9.87±0.94	9.92±0.97	9.90±0.96	9.93±0.97

Values shown are mean ± SD (standard deviation)
[a,b] means in a row with different superscript differ significantly (P<0.05)

3.3 Albumen quality parameters

The results of egg quality determination are presented in Table 3. The Haugh unit and the albumen height of the HS groups were consistently higher (P<0.05) compared to the control. Among treatments, significant variations (P<0.05) in the albumen height and albumen width were found. No statistical differences (P≥0.05) were found for the albumen weight and the albumen percentage among the treatment groups. Also, Macit et al. (2009) [3] and Arpášová et al. (2016) [20] found that HS exerted a positive effect on albumen index and HU. In contrast, some reports documented that the application of HS to the diet or water of laying hens had no effect on HU [1,2,27,29], albumen height [18,27,29] and albumen percentage [18,27].

3.4 Yolk quality parameters

Addition of HS in different concentrations significantly increased (P<0.05) yolk height, yolk width and yolk index.

There were no statistically significant differences (P>0.05) among the control and experimental treatment groups in terms of yolk weight and yolk color. The yolk percentage of the control group was significantly higher (P<0.05) compared to HS treatments throughout the study. The yolk weight of was no significantly affected (P≥0.05) by HS addition. The results were in contrast with those reported by Kaplan et al. (2018).

In comparison, Yalcin et al. (2006) [27] recorded that the addition of HS no effect on yolk percentage. However, Hanafy and El-Sheikh (2008) [18] found that HS caused a decrease in egg yolk percentage. In accordance with Yörük et al (2004) [22], we reported no effect of HS dietary supplementation on yolk colour.

4. Conclusions

These results of this study showed that the supplementation of HS in different into layer diet can affected some parameters of eggs quality. Further studies of the basic mechanisms of HS depending on their origin, dose and method of application involved in egg quality are needed.

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