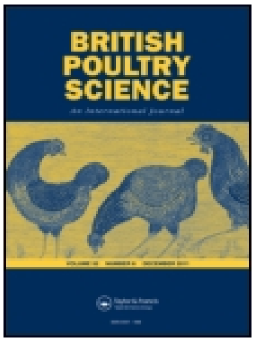

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E. Ozturk, I. Coskun, N. Ocak, G. Erener, M. Dervisoglu & S. Turhan

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Performance, meat quality, meat mineral contents and caecal microbial population responses to humic substances administered in drinking water in broilers

E. OZTURK, I. COSKUN[1], N. OCAK, G. ERENER, M. DERVİSOGLU[2], AND S. TURHAN[2]

Department of Animal Science, Faculty of Agriculture, Ondokuz Mayis University, 55139 Samsun, Turkey, [1]Department of Animal Science, Faculty of Agriculture, Ahi Evran University, Asikpasa, 40000 Kirsehir, Turkey, and [2]Department of Food Engineering, Faculty of Engineering, Ondokuz Mayis University, 55139 Samsun, Turkey

Abstract 1. This study was conducted to examine the effect of different levels of humic substances (HS) administered in drinking water on caecal microflora and mineral composition and colour characteristics of breast and thigh meats and the growth performance, carcass and gastrointestinal tract (GIT) traits of broiler chicks.

2. A total of 480 3-d-old broiler chickens were randomly allocated to 4 treatments with 4 cages per treatment and 30 bird (15 males and 15 females) chicks per cage. All birds were fed on commercial basal diet. The control birds (HS0) received drinking water with no additions, whereas birds in the other treatment groups received a drinking water with 7.5 (HS7.5), 15.0 (HS15.0) and 22.5 (HS22.5) g/kg HS. Mush feed were provided on an ad libitum basis. Body weight and feed intake of broilers were determined at d 0, 21, and 42, and feed conversion ratio was calculated. On d 42, 4 broilers (2 males and 2 females) from each cage were slaughtered and the breast and thigh meats were collected for mineral composition and quality measurements.

3. Performance, carcass and GIT traits and caecal microbial population of broiler chicks at d 42 were not affected by the dietary treatments. The lightness (L^*) of breast and thigh meat decreased in broilers supplemented with 15 and 22.5 g/kg HS in drinking water. Although the redness (a^*) of breast meat increased, yellowness of thigh meat decreased in broilers supplemented with 15 and 22.5 g/kg HS in drinking water ($P < 0.05$).

4. In conclusion, the 15 and 22.5 g/kg HS administration in drinking water can be applied for broiler chicks to maintain growth performance and improve meat quality without changing caecal microflora.

INTRODUCTION

The optimum growth and economical feed conversion as well as prevention and control of diseases are dependent on the feed additives (Ozturk *et al.*, 2012). There is a large variety of feed additives, including organic acids such as acetic, propionic, butyric, formic, citric, fumaric, lactic, malic and humic acids or commercial acid blends (Islam *et al.*, 2005, 2008; Esenbuğa *et al.*, 2008; Ocak *et al.*, 2009; Ozturk *et al.*, 2010, 2012), that can be used to replace antibiotic growth promoters. Humic substances (HS) are major components of the natural organic matter in soil and water as well as in geological organic deposits, such as lake sediments, peats, brown coals and shales (Islam *et al.*, 2005). The HS, humic, fulmic and ulvic acids are not antibiotics but, if used correctly along with nutritional, managerial and bio-security measures, they can be a powerful tool in maintaining the health of the gastrointestinal tract (GIT) of poultry, thus improving their performances (Kocabagli

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Correspondence to: E. Ozturk, Ondokuz Mayis Universitesi, Ziraat Fakultesi, Zootekni Bolumu Kurupelit, 55139 Samsun, Turkey.
E-mail: eozturk@omu.edu.tr

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et al., 2002; Ceylan *et al.*, 2003; Windisch *et al.*, 2008). Most of the data on humic, fulmic, ulvic acids and humin refer to average properties and structure of a large ensemble of components of diverse structure and molecular weight (Islam *et al.*, 2005; Ozturk *et al.*, 2010, 2012). The properties of HS are well documented (Peña-Méndez *et al.*, 2005; Islam *et al.*, 2005; Ji *et al.*, 2006).

Studies investigating the effects of the HS and humic acids administration in the diet or drinking water on live weight, feed consumption and characteristics of carcass and GIT in broiler chickens (Rath *et al.*, 2006; Ozturk *et al.*, 2010, 2012; Šamudovská and Demeterová, 2010) indicated that HS improved protein digestion and trace element utilisation (Huang *et al.*, 1994; Yang *et al.*, 1996,), and it has a positive influence on growth rate (Shermer *et al.*, 1998; Eren *et al.*, 2000; Kocabagli *et al.*, 2002; Ceylan *et al.*, 2003). We found that the HS supplementation at levels of 300 and 450 g/kg in drinking water appears to have a measurable impact on live performance by improving feed efficiency and lightness of breast and thigh meat in broilers, respectively (Ozturk *et al.*, 2010). Also we found that feeding with a diet containing HS caused a measurable variation in the meat quality and blood cholesterol as well as the performance, carcass and GIT traits of broilers. The antimicrobial effects of HS, including humic acid, have been demonstrated, but the reports on their influence on growth performance of poultry are variable; therefore, it needs more investigation (Ozturk *et al.*, 2010, 2012). On the other hand, little is known on whether humic acid shows an antimicrobial effect against opportunistic pathogens existing in GIT (Islam *et al.*, 2008). Thus, the aim of this study was to evaluate the effects of different doses of HS supplementation provided through drinking water on growth performance, characteristics of carcass and gut, gut microflora and colour characteristics of breast and thigh meat in broilers.

Table 1. *Ingredients and nutrient composition of diets*

	Starter (1 to 21 d)	Grower (22 to 35 d)	Finisher (36 to 42 d)
Ingredients (g/kg)			
Yellow maize	408.6	330.0	416.0
Soybean meal	290.3	276.2	250.0
Sunflower meal	77.0	0.0	0.0
Cracked wheat	100.0	65.0	125.0
Wheat bran	0.0	200.0	100.0
Meat and bone meal	64.0	64.0	51.3
Vegetable oil	52.0	56.2	50.0
Sodium chloride	2.3	2.3	2.4
Vitamin and mineral premix[1]	3.5	3.5	2.5
L-Lysine	1.2	1.2	1.2
DL-Methionine	1.1	1.6	1.6
Calculated nutrition composition (g/kg)			
ME, MJ/kg	13.0	13.4	13.4
Dry matter	890.0	887.0	889.0
Crude protein	230.0	210.0	190.0
Ca	10.1	10.0	8.0
Available P	5.0	4.8	4.4

[1] Supplied per kg diet: trans-retinyl acetate 12 000 IU, cholecalciferol 2400 IU, DL-α-tocopheryl acetate 40 mg, menadione 4 mg, thiamine 3 mg, riboflavin 6 mg, nicotinic acid 25 mg, folic acid 1 mg, calcium-D-pantothenat 10 mg, pyridoxine 5 mg, cyanocobalamin 0.03 mg, D-biotin 0.05 mg; manganese 80 mg, zinc 60 mg, iron 60 mg, copper 5 mg, cobalt 0.2 mg; iodine 1 mg, selenium 0.15 mg, choline chloride 200 mg.

MATERIALS AND METHODS

For the trial, 480 3-d-old broiler chicks (ROSS 308) were allocated into 4 groups (HS0, HS7.5, HS15 and HS22.5) of 120 mixed-sex birds. Each treatment was divided into 4 replicates of 30 chicks (15 females and 15 males). All birds were fed ad libitum the same antibiotic-free commercial diet for the starter (from d 1 to d 21), grower (from d 22 to d 35) and finisher (from d 36 to d 42) periods (Table 1). All animals in experimental treatments were housed in floor pens with wood shavings and fed on the same basic diets. The control birds (HS0) received drinking water with no additions. Birds in the other treatment groups received a drinking water with 7.5 (HS7.5), 15.0 (HS15.0) and 22.5 (HS22.5) g/kg HS per kg of body weight. The liquid HS, measured for each of treatments, was added into a sufficient amount of drinking water based on estimated water consumption recommended by the producer company. Therefore, drinking water was prepared daily for each of the treatments and also to ensure consumption of HS-treated water; the treated water up to the half of daily water requirement were given by plastic poultry drinker. After all of the HS-treated water was consumed, birds were watered ad libitum by automatic drinkers. Thus, daily water intake was not measured. The HS used in present study was reported to include 4.9% dry matter, 61.2% humic acid, 5.1% fulvic acid, 7.53% crude protein, 0.48% crude fibre, 2.35% ether extract, 2.49% Ca, 0.24% Mg, 0.15% K, 1.09% Na, 1.49% S, 0.07% P, 0.28% NO_3, 1.89% Fe, 0.05% Zn, 0.02% Cu, 0.01% Ni, 0.02% Cr, respectively (Ozturk *et al.*, 2010).

During the trial, continuous lighting was provided throughout the experiment. Ambient temperature was gradually decreased from 33°C on d 7 to 21°C on d 21 and was kept constant. All the cages were checked for mortality twice a day and mortality was recorded as it occurred and was used to adjust the total number of birds to determine the total feed intake per bird and feed conversion ratio. Live weight and feed intake were measured at 1, 21, 35 and 42 d of age. The birds were weighed and fed as mixed sex in each group. Therefore, the chick weight, the daily weight gain and the feed efficiency was not determined per sex. At 42 d of age, birds were starved for 6 h before slaughtering, and 4 birds (2 females and 2 males) per replicate or 16 birds per treatment were slaughtered (Ozturk *et al.*, 2010, 2012). Plucked and eviscerated carcasses were weighed after removal of the head, neck, feet and abdominal fat to obtain ready-to-cook carcasses that were refrigerated for 6 h at 4°C. Yields from chilled carcasses, breast and thighs were evaluated. Breast (*Pectoralis major*) and thigh (*Iliotibialis*) meat samples were vacuum-packaged and kept frozen (−20°C) until chemical analyses were performed.

The Commission Internationale de l'Éclairage (CIE) colour values of meats (lightness (L^*), redness (a^*) and yellowness (b^*)) were measured at 8 h post mortem using a Minolta CR 300 Chroma Meter (Minolta Camera Co., Osaka, Japan). Four replicate measures were performed on breast and thigh meats, respectively, representing the whole surface of the muscles, and mean colour values were calculated for each sample. The colorimeter was calibrated throughout the study using a white and pink ceramic tile. A white tile (L^* = 92.30, a^* = 0.32 and b^* = 0.33) was used as standard. Lightness may range from 100 (white) to 0 (black). While positive a^* and b^* values are a measure of redness and yellowness, respectively, negative a^* and b^* values indicate greenness and blueness. Mineral composition of breast and thigh meat was determined with atomic absorption spectrophotometer, except *P* that was analysed with an atomic absorption spectrophotometer (Horuz and Korkmaz, 2006).

Pre-weighed ileum samples (1 g) for microbiological analyses were transferred into dilution bottles. Anaerobic diluents were added to achieve a 1 to 10 (w/v) dilution. The samples were mixed with a vortex until completely suspended and dispensed using standard methods into a 1 to 10 (v/v) dilution series of tubes containing anaerobic peptone buffer. Appropriate dilutions were inoculated onto the plates. The following media and incubation conditions were used to enumerate microbial counts of samples: Rogosa agar for *lactobacilli* at 30°C for 5 d, Plate Count agar for aerobic mesophilic bacteria at 30°C for 48 h, Violet-Red Bile agar for coliform bacteria at 35°C for 24 h, M17 agar for *lactococci* at 30°C for 72 h and Chromocult TBX agar for *Escherichia coli* (*E. coli*) at 44°C for 24 h.

For performance data, pen means served as the experimental unit for statistical analysis. For data of relative weights and length of gut, meat quality traits, chemical composition, blood parameters and caecal microbial population, slaughtered individual birds were considered as the experimental unit. All percentage data were transformed by taking arcsine square roots before analysis. To evaluate statistically the measured data, one-way analysis of variance was performed in a completely randomised design: $\hat{Y}_{ij} = \mu + \alpha_i + e_{ij}$ ($\hat{Y}_{ij}$, observation values (body weight gain, feed consumption, feed to gain, carcass traits, colour measurements, blood parameters, etc.); μ, the overall mean; α_i, the effect of the ith treatment ($i = 1,\ldots, 4$; HS0, HS7.5, HS15.0 and HS22.5) and e_{ij}, residual error). Tukey's test was used to determine the effect of treatments and differences which were considered to be significant at $P < 0.05$.

RESULTS

No significant differences in mortality among the treatment groups (0.83%, 1.67%, 0.83% and 0.83% for the HS0, HS7.5, HS15 and HS22.5, respectively) were observed. Daily weight gain, daily feed intake and feed efficiency of broilers receiving the HS supplemented drinking water are presented in Table 2. The daily weight gain, daily feed intake and feed efficiency were not affected by the treatments (P > 0.05). Therefore, HS administered in drinking water did not have any harmful effects on performance and had no growth-promoting effect compared to control on broilers.

Means for carcass weight, dressing percentage, the relative weight and length of gut and the relative weight of edible inner organs (such as gizzard, heart, liver) and abdominal fat pad at 42 d of age are shown in Table 3. There were no differences among the experimental groups compared to the control group in terms of the carcass weight, dressing percentage, the relative weight and length of gut and the relative weight of edible inner organs and abdominal fat pad (P > 0.05).

The L^* and a^* values of breast and L^* and b^* values of thigh meat were affected by HS supplementation (Table 4), the L^* values of breast meat

Table 2. *Initial body weight, daily weight gain, daily feed intake and feed efficiency of broilers receiving humic substances in drinking water*

	HS0	HS7.5	HS15	HS22.5	SEM	*P*-value
Initial body weight, g per bird	50.0	49.8	49.9	50.0	0.07	NS
Daily weight gain, g per bird	54.1	54.1	54.2	54.4	0.67	NS
Daily feed intake, g per bird	95.3	96.4	95.2	94.9	0.67	NS
Feed efficiency, g feed:g gain	1.76	1.78	1.76	1.74	0.01	NS

Data represent the mean value of 4 replicate pens of 30 birds.
SEM, standard error of mean.
NS, $P > 0.05$.

Table 3. *Carcass weight, dressing percentage and cut-up parts of broilers receiving humic substances in drinking water (mean, n = 16)*

	HS0	HS7.5	HS15	HS22.5	SEM	*P*-value
Carcass weight (g)	1609	1611	1634	1642	20.3	NS
Dressing percentage (%)	70.84	70.88	71.79	71.90	0.488	NS
Relative weight of (g/100 g body weight)						
Whole gut	8.71	8.72	8.75	8.80	0.243	NS
Empty gizzard	1.97	2.08	2.16	2.27	0.056	NS
Heart	0.64	0.65	0.66	0.67	0.014	NS
Liver	2.58	2.72	2.75	2.82	0.050	NS
Abdominal fat	2.72	2.81	2.99	3.16	0.157	NS

SEM, standard error of mean.

NS, $P > 0.05$.

Table 4. *Colour measurements of breast and thigh meats of broilers receiving humic substances in drinking water (mean, n = 16)*

	HS0	HS7.5	HS15	HS22.5	SEM	*P*-value
Breast meat						
L^*, lightness	54.71^{a}	53.88^{ab}	53.08^{b}	52.88^{b}	0.513	*
a^*, redness	3.47	3.61	3.67	3.81	0.075	NS
b^*, yellowness	2.57^{a}	1.84^{b}	1.75^{b}	0.36^{c}	0.100	*
Thigh meat						
L^*, lightness	47.94^{a}	49.04^{a}	46.18^{b}	46.04^{b}	0.278	*
a^*, redness	1.55^{a}	2.02^{b}	2.06^{b}	2.08^{b}	0.097	*
b^*, yellowness	2.72	2.28	2.17	1.94	0.249	NS

^{a,b,c}Mean values within the same row sharing a common superscript letter are not statistically different at $P < 0.05$.

SEM, standard error of mean.

*$P < 0.05$; NS, $P > 0.05$.

were higher at HS0 than that of HS15 and HS22.5 groups ($P < 0.05$). The b^* values of breast meat from 22.5HS birds were lower than those from other treatment birds ($P < 0.05$). The L^* values of thigh meat was higher in control and HS7.5 birds than those in other treatments ($P < 0.05$). The thigh meat from all of the HS treatment groups had a higher a^* value compared to the HS0 ($P < 0.05$).

Mineral composition of breast and thigh meats of broilers receiving the HS-supplemented drinking water is presented in Table 5. Percentages of Ca, Mg, K, Na, P, Fe and Cu of breast meat were not affected by the dietary treatments, but content of Zn increased by HS7.5 in respect to 0HS. Percentages of Ca, Mg, K, Na, Cu and Zn of thigh meat were not affected by the dietary treatments, but percentage of *P* was higher in HS7.5 compared to HS15, whereas Fe level was higher in HS22.5 compared to HS7.5.

Bacterial counts ($\log_{10}$ cfu/g) from the caecal of broilers receiving HS-supplemented drinking water are presented in Table 6. Aerobic mesophiles, *lactococci, lactobacilli,* coliforms and *E. coli* counts in the caecum were not affected by HS in drinking water (Table 6).

Table 5. *Mineral composition (ppm on fresh matter) of breast and thigh meats from broilers receiving humic substances in drinking water (mean, n = 16)*

	HS0	HS7.5	HS15	HS22.5	SEM	*P*-value
Breast meat						
Ca	7.4	16.9	7.0	5.9	0.20	NS
Mg	42.3	49.5	40.8	39.8	0.27	NS
K	258.6	279.5	243.1	247.4	1.16	NS
Na	8.7	10.0	11.8	10.0	0.06	NS
P	94.2	101.5	96.1	90.2	0.23	NS
Fe	0.7	0.7	1.2	0.9	0.01	NS
Cu	0.1	0.1	0.1	0.2	0.02	NS
Zn	0.6^{a}	0.8^{b}	0.7^{ab}	0.7^{ab}	0.03	*
Thigh meat						
Ca	4.6	4.6	6.2	6.8	0.49	NS
Mg	40.8	44.0	40.5	40.8	1.50	NS
K	280.1	281.6	288.0	258.7	8.39	NS
Na	8.8	9.3	9.0	9.8	0.45	NS
P	98.9^{ab}	101.7^{b}	90.4^{a}	97.3^{ab}	1.67	*
Fe	1.3^{ab}	0.7^{a}	1.0^{ab}	1.9^{b}	0.19	*
Cu	0.3	0.2	0.2	0.2	0.04	NS
Zn	0.7	0.6	0.6	0.7	0.04	NS

^{a,b,c}Mean values within the same row sharing a common superscript letter are not statistically different at $P < 0.05$.

SEM, standard error of mean.

*$P < 0.05$; NS, $P > 0.05$.

Table 6. *Bacterial counts ($\log_{10}$ cfu/g) from the caecal content of broilers receiving humic substances in drinking water (mean, n = 16)*

	HS0	HS7.5	HS15	HS22.5	SEM	*P*-value
Aerobic mesophiles	8.65	8.55	8.74	8.30	0.090	NS
Lactococci on M17	7.53	7.53	7.62	7.28	0.120	NS
Lactobacilli on Rogosa	7.72	8.33	7.40	7.94	0.170	NS
Coliforms	7.51	6.91	6.91	7.15	0.110	NS
E. coli	6.74	6.66	6.58	6.38	0.097	NS

SEM, standard error of mean.

NS, $P > 0.05$.

DISCUSSION

The results of the present study indicated that the 15 and 22.5 g/kg HS administration in drinking water can be applied for broiler chicks to maintain growth performance, without changing caecal microflora, and to improve meat quality. In the present study, mortality was low and within the accepted limit for all groups, and the deaths were not associated with any specific treatment. Our result could be considered as similar to our previous observations (Ozturk *et al.*, 2010, 2012). Likewise, Rath *et al.* (2006) and Kocabagli *et al.* (2002) found no differences in mortality in their study. It can be said that the potential interactions of HS with commonly occurring pathogens or environmental stress can hardly be evaluated under the controlled conditions. Therefore, it is considered that the cause of death of chickens in control and HS groups was the sudden death syndrome, conforming to the results of studies by Yoruk *et al.* (2004), Karaoglu *et al.* (2004) and Islam *et al.* (2008). The fact that HS-treated water did not induce deterioration in feed intake and weight gain in our present and previous study (Ozturk *et al.*, 2010) indicate that HS given in drinking water did not affect water intake. Indeed, we observed that, in the both experiments, birds consumed voluntarily all of the HS-treated water.

External appearance (colour) of meat and consistency of colour as well as water-holding capacity and texture are important meat quality characteristics that can affect consumer preferences (Fletcher, 2002; Qiao *et al.*, 2002). Moreover, there is a relationship among some meat quality parameters such as colour attributes (CIE L^*, a^* and b^*), pH and water-holding capacity (Fletcher, 2002). Colour of meat is not only a quality characteristic, but is also an indicator of animal health, related directly to stress and energy metabolism (Nijdam *et al.*, 2005; Kop and Ocak, 2009). Therefore, one of the primary aims of the present study was to investigate the changes in colour characteristics and chemical compositions of both breast and thigh meats. Yoruk *et al.* (2004) have demonstrated that darkening in thigh muscles and an increase in their redness might indicate an increase in haem pigments because the red colour in meat is due mainly to a protein pigment called myoglobin and, to a lesser extent, haemoglobin. Ozturk *et al.* (2010) have reported that inclusion of 0.5 g/kg HS via water enhanced a^* values of broiler breast and thigh meat, and Ozturk *et al.* (2012) have hypothesised that the increase of a^* values of breast and thigh meat might result from increased iron content of breast and thigh meats. Although there was no statistical difference among the groups in terms of Fe percentage of breast meat, HS22.5 tended to increase Fe content of thigh meat compared to HS7.5. HS15 and HS22.5 increased redness of breast meat. Although the L^* and a^* values of breast meat and the L^* and b^* values of thigh meat decreased by high levels of HS administration, the colour characteristics for all treatments in the current study were within the normal range (Qiao *et al.*, 2002; Ozturk *et al.*, 2010, 2012). Thus, the meats and the changes in colour attributes would not be considered excessively pale or dark and favourable, respectively. Therefore, our results confirmed suggestions by Ozturk *et al.* (2012).

The changes of Zn, P and Fe concentrations in meats by HS administration in drinking water may be due to metal chelating effects of humic acids (Rath *et al.*, 2006; Šamudovská and Demeterová, 2010) that are affected by a large number of carboxylic acid side chains. Our results with respect to other mineral concentrations in meats are in general agreement with a previous study (Avci *et al.*, 2007), in which humic acid was used in broiler chicken diets. Comparing results of studies by research worldwide, performance differences due to humate supplementation might result from the compositional differences among the commercially available humate products (Kocabagli *et al.*, 2002; Ozturk *et al.*, 2010, 2012; Šamudovská and Demeterová, 2010).

Our results with respect to the weight gain, feed intake and feed efficiency are in agreement with a previous study (Eren *et al.*, 2000) using humic acid in broiler diets. However, Kocabagli *et al.* (2002) found that adverse effects disappear promptly after 2 weeks. These authors agreed that feeding humic acid to broilers had a growth-promoting effect only during the later stage of growth (22–42 d). Therefore, our results support these results because of the recovering tendency observed in body weight gain after 2 weeks of rearing. Although the caecal microbial count was not affected by HS in drinking water in the present study, Shermer *et al.* (1998) and Ceylan *et al.* (2003) indicated that the humic acid is an alternative to antibiotic growth promoters in broiler diets by altering the microflora in the gastrointestinal system, especially in the caecum. Therefore, the previous findings related to humic acid (Kocabagli *et al.*, 2002; Ceylan *et al.*, 2003; Esenbuğa *et al.*, 2008; Windisch *et al.*, 2008; Aksu and Bozkurt, 2009; Šamudovská and Demeterová, 2010) are somewhat contradictory and not helpful in interpreting our data regarding growth, feed intake and feed efficiency. Eren *et al.* (2000) also indicated insignificant changes in feed conversion efficiency because of continuous addition of humic acid.

The fact that HS-treated water did not affect the daily weight gain and feed efficiency might result from doses of HS administered or lacking of stress factor in the present study. Indeed, broilers in our previous studies (Ozturk *et al.*, 2010, 2012) and in the control group of the present study had a fairly high body weight gain compared to the birds that received the HS-treated water. Responses to alternatives to antibiotic growth promoters may be greater in a more challenging environment (Ocak *et al.*, 2009; Ozturk *et al.*, 2010, 2012). According to these findings, it can be said that feed additives such as humic acids or humates (Midilli *et al.*, 2008) are not effective if there are no stress factors. The result with respect to mortality showed that broilers in the present study were kept under clean and comfortable environmental conditions. It was reported that humic acids stabilise the intestinal flora and thus ensure an improved utilisation of nutrients in animal feed (Islam *et al.*, 2005). Aksu and Bozkurt (2009) have reported that 150 g/kg humic acid inclusion into broiler feed decreased the *E. coli* count. On the contrary, there was no change among the groups with regard to intestinal microflora in this study; however, *E. coli* count in ileum samples tended to decrease.

The result of the present study indicate that 7.5, 15 and 22.5 g/kg HS per bird provided through drinking water appears to have no measurable impact on growth performance, feed efficiency and caecal microflora, but have a measurable impact on lightness, redness and yellowness of breast and thigh meat colours in broilers, respectively. The data presented on the effects of HS in chickens for fattening would not suffice to encourage producers or authorities for use of HS as feed additive. In conclusion, it has been shown that inclusion of HS into broiler drinking water has no positive effect on broiler performance parameters, gut microflora and mortality but, inclusion of 15 and 22.5 g/kg HS decreased lightness of breast and thigh meat and increased redness of breast meat and decreased yellowness of thigh meat. Further research should focus on the identification of optimal concentrations of feed additive and feeding strategy.

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