Effect of Diet Supplemented with Natural Humic Compounds and Sodium Humate on Performance and Selected Metabolic Variables in Broiler Chickens

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Abstract

The effect of humic substances from different sources on the performance and selected biochemical indicators was studied in 150 one-day-old broiler chickens (Ross 308) divided into control (C) and two tested groups (n = 50). Chickens of tested groups were fed diets supplemented with natural humic compounds (group HS) and sodium humate (group HNa) at amounts of 5 g·kg⁻¹ of feeds in phase 1 and 7 g·kg⁻¹ of feeds in phases 2 and 3 of the fattening period. Higher final body weights (not significant) were observed in both tested groups (2527.6 g for HS; 2481.5 g for HNa) than in the control group (2476.6 g). The feed conversion ratio throughout the whole experiment was lower in the HS group (P < 0.001) and higher in the HNa group (P < 0.001) compared to the control group. The European Efficiency Index reached at the end of the experiment was the highest in the HS group (P < 0.001) and the lowest in the HNa group compared to the control group. Differences in total protein, albumin, total lipids, triglycerides and phosphorus among the tested groups were not significant. Higher values of Ca (P < 0.05) in the HS group and lower cholesterol (P < 0.05) in the HNa group were observed after 14 days; lower AST (P < 0.05) in the HS group and lower calcium (P < 0.05) and higher glucose (P < 0.01) in the HNa group were observed after 35 days of the experiment in comparison with the control group.

Humic substances, production, biochemical indices

Humic substances are natural, yellow to brown-black coloured organic compounds with a relatively high molecular weight ranging from 2 to 200 kDa, that originate from the decomposition of plant and animal remains (Stevenson 1994). This process of decomposition is called humification. Humic substances can be found in soil, peat, lignites, brown coals, sewage, natural waters and their sediments. The content of humic substances in these materials varies from trace amounts in sands and clays to tens of per cents (3-10%) in brown coal. Extremely high amount (up to 85%) is present in the lignite, the peat and the oxyhumolite (oxidised brown coal) (Peña-Méndez et al. 2005; Skokanová and Dercová 2008). Humic substances contain humin, humic acid, fulvic acid, ulmic acid and some microelements (Stevenson 1994) such as iron, manganese, copper and zinc (Aiken et al. 1985). Nowadays, humic substances are used in agriculture, industry (building industry, woodworking industry, ceramic industry and paper industry), environmental and bio-medicine. In agriculture they are used mainly as fertilizers in the form of humates to increase soil fertility, to increase transfer of micronutrients from soil to plants, to increase seed germination rates, and to reduce the usage of mineral fertilizers (Peña-Méndez et al. 2005). The main function of humic substances in environmental chemistry is to reduce the toxic effect of residual amounts of heavy metals, herbicides, fungicides, insecticides, nematicides, dioxins and radionuclides and other harmful substances in water and soil (Shin et al. 1999; Loffredo et al. 2000; Kucukersan et al. 2005). In the veterinary medicine they are used in horses, ruminants, swine and poultry for the treatment of diarrhoea, dyspepsia and acute intoxications. They have a protective action on the mucosa of the intestine via a protective film formation (Kucukersan et al. 2005). They can also bind
toxic metals and other toxic substances by the formation of insoluble and non-resorbable complexes (Alvarez-Puebla et al. 2004) and have antiphlogistic and antimicrobial properties (EMEA 1999). In recent years the interest in the use of humic substances in animal husbandry has increased. Many authors in their studies observed an improvement in growth and feed conversion, and reduction of animal mortality after addition of humic substances into feedstuff (Eren et al. 2000; Kocabağli et al. 2002; Karaoglu et al. 2004; Ji et al. 2006; El-Husseiny et al. 2008). An improvement in egg production and egg weight was observed in a study of the effect of humic acids in laying hens performed by Kucukersan et al. (2005). Although there is not enough evidence to hypothesize how humates promote growth, it is assumed that humates might increase the uptake of nitrogen, phosphorus and other nutrients due to their chelating properties (Kocabağli et al. 2002).

The objective of the present study was to investigate the effect of supplementation of natural humic compounds and sodium humate on the performance and selected biochemical indicators of broilers.

**Materials and Methods**

**Birds and diets**

In this study, a total of 150 unsexed one-day-old broiler chickens (Ross 308) obtained from a commercial supplier were used. Chickens were weighed, randomly divided into three groups: one control (C) and two tested groups (HS, HNa) with 50 chicks per group. Birds were housed on deep bedding in agreement with the technological instruction for Ross 308 chickens, with controlled light, temperature, animal hygiene and feeding regime. Birds were fed a complete mixture in the mash form according to the growth phases (phase 1: week 1 to 2; phase 2: week 3 to 5; phase 3: week 6) ad libitum. The composition of feed mixtures is shown in Table 1. The tested group diets were supplemented with natural humic compounds (oxyhumolite - total humic acids 68%, free humic acids 48%, minerals 18%; locality Dudar, Hungary) (group HS) and sodium humate (dry matter 84.8%, humic acids 63.2%, ash 36.9% in dry matter) (group HNa) at different amounts: 5 g·kg⁻¹ of diet during first two weeks and 7 g·kg⁻¹ of diet from week 3 to 6. No antibiotic growth promoters or anticoccidials were used in the diets.

Birds were individually weighed and the feed consumption was observed weekly. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated.

Mortality was recorded as it occurred and percentage of mortality was determined on day 35 and at the end of the study. The following equation was used for the evaluation of results using European Efficiency Index (EEI):

\[
EEI = \frac{\text{live weight (kg) } \times \text{liveability} \times 100}{\text{age (days) } \times \text{feed conversion}}
\]

Blood samples were collected from ten birds in each group on days 14 and 35 of experiment from the jugular vein for biochemical analysis.

The experiment was carried out in the barns of the Institute of Animal Nutrition and Dietetics at the University of Veterinary Medicine in Košice in compliance with the EU regulations concerning the protection of experimental animals. The experiment was carried out with the consent of the institutional Animal Care and University Ethics Committee.

**Analytical methods**

Diets were analyzed for dry matter, crude protein, ether extract, crude fibre and ash by the AOAC (2001).

Total protein, albumin, glucose, total lipids, cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase, calcium and phosphorus blood serum concentrations were determined by spectrophotometry using commercial Bio-La-Tests (Pliva-LaChema Brno Ltd., Czech Republic).

**Statistical analysis**

Statistical evaluation of the effects of natural humic compounds and sodium humate on body weight, feed conversion ratio, European Efficiency Index and biochemical indices of chickens among the groups was performed by one-way ANOVA (analysis of variance) and significance of mean differences between the groups was tested by Tukey-Kramer multiple comparison test (level of significance set at \( P < 0.05 \), \( P < 0.01 \), \( P < 0.001 \)).

**Results and Discussion**

Composition, nutrient and metabolizable energy content of diets for the control and tested groups used in experimental periods are shown in Table 1. Diets used in the control and tested groups in respective periods were isoenergetic and isonitrogenous.

There were no significant differences in the initial body weights of chicks between
groups at the beginning of the experiment (C 41.5 ± 0.5 g; HS 40.8 ± 0.5 g; HNa 40.9 ± 0.5 g). Birds in both tested groups had higher final body weights than birds in the control group (Table 2). The highest final body weight was found in the group of birds fed the diet with natural humic compounds (HS), but with no significant differences between groups. The body weights of chickens in both tested groups were lower compared to the control group in the individual weeks of the experiment until week 5. Significant differences were observed between the HNa group and the control group after the first week of the experiment (10.3 %; \( P < 0.01 \)). The average body weight gain and feed conversion ratio values in respective phases are shown in Table 3. The average BWG was lower in both tested

### Table 1. Composition, nutrient and metabolizable energy content of diets

<table>
<thead>
<tr>
<th>Ingredients (g·kg(^{-1}))</th>
<th>Week 1–2</th>
<th>Week 3–5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>HS</td>
<td>HNa</td>
<td>C</td>
</tr>
<tr>
<td>Maize</td>
<td>435</td>
<td>435</td>
<td>435</td>
</tr>
<tr>
<td>Wheat</td>
<td>121</td>
<td>116</td>
<td>116</td>
</tr>
<tr>
<td>Soybean meal (45%)</td>
<td>360</td>
<td>360</td>
<td>360</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Limestone</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Vitamin-mineral premix(^{1,2,3})</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Lysine</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Natural humic compounds(^4)</td>
<td>–</td>
<td>5.0</td>
<td>–</td>
</tr>
<tr>
<td>Sodium humate</td>
<td>–</td>
<td>–</td>
<td>5.0</td>
</tr>
<tr>
<td>Analysis (g·kg(^{-1})DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry mater</td>
<td>896.9</td>
<td>895.6</td>
<td>898.3</td>
</tr>
<tr>
<td>Crude protein</td>
<td>249.9</td>
<td>251.2</td>
<td>249.4</td>
</tr>
<tr>
<td>Ether extract</td>
<td>70.1</td>
<td>69.5</td>
<td>72.3</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>36.7</td>
<td>37.1</td>
<td>40.2</td>
</tr>
<tr>
<td>Ash</td>
<td>82.3</td>
<td>81.7</td>
<td>73.7</td>
</tr>
<tr>
<td>ME (MJ·kg(^{-1})DM)</td>
<td>13.3</td>
<td>13.3</td>
<td>13.3</td>
</tr>
</tbody>
</table>

C - control group; HS - group with natural humic compounds; HNa - group with sodium humate; DM - Dry matter; ME - Metabolizable energy

\(^{1,2,3}\) mineral-vitamin premix (per kg) 1 – Ca 95 g, P 135 g, Na 75 g, Mg 5 g, DL-methionine 80 g, vit.A 600,000 IU, D\(_3\) 135,000 IU, E 900 mg, K\(_3\) 150 mg, panthotenic acid 600 mg, niacin 4000 mg, cholin chloride 20,000 mg, B\(_2\) 150 mg, B\(_3\) 900 µg, biotin 3000 µg, folic acid 76,000 µg, vit. C 2000 mg, Fe 1500 mg, Cu 500 mg, Zn 3000 mg, Mn 5000 mg, I 25 mg, Se 23 mg, Co 10 mg.

\(^2\) – Ca 100 g, P 135 g, Na 75 g, Mg 5 g, DL-methionine 80 g, vit. A 425,000 IU, D\(_3\) 84,000 IU, E 900 mg, K\(_3\) 100 mg, pantotenic acid 420 mg, niacin 3400 mg, cholin chloride 14,200 mg, B\(_2\) 100 mg, B\(_3\) 640 µg, biotin 2150 µg, folic acid 54,500 µg, vit.C 1400 mg, Fe 1500 mg, Cu 500 mg, Zn 3000 mg, Mn 5000 mg, I 25 mg, Se 23 mg, Co 10 mg.

\(^3\) – Ca 110 g, P 145 g, Na 75 g, Mg 9 g, DL-methionine 55 g, vit. A 370,000 IU, D\(_3\) 135,000 IU, E 900 mg, K\(_3\) 95 mg, panthotenic acid 370 mg, niacin 3880 mg, cholin chloride 14,000 mg, B\(_2\) 95 mg, B\(_3\) 560 µg, biotin 1850 µg, folic acid 47,000 µg, vit.C 1240 mg, Fe 1500 mg, Cu 500 mg, Zn 3000 mg, Mn 5000 mg, I 25 mg, Se 23 mg, Co 10 mg

\(^4\) – oxyhumolite - (locality Dudar, Hungary)
groups than in the control group throughout phase 1 of the study. The lowest BWG was observed in the HNa group. Throughout phase 2 the highest BWG were in the HS group and the lowest in the HNa group. Both tested groups had higher BWG than the control group in phase 3. The highest BWG were observed in the HS group. Considering the whole experiment period the BWG were higher in both tested groups than in the control group (HS 2486.8 g, HNa 2440.8 g, C 2435.1 g) (Fig. 1). The most intensive growth rate was noticed in chickens of the HS group. Noticeably higher intensity of growth was observed in both tested groups from week 4 of the experiment.

While in phase 1 the FCR in the HS group was higher ($P < 0.01$) and in phase 2 similar to control, in phase 3 it was significantly lower ($P < 0.01$). The FCR was higher in the HNa group in all phases of the study (significantly in phase 2, $P < 0.01$) compared to control. The FCR throughout the whole experimental period was significantly lower in the HS group (1.69 kg·kg$^{-1}$ BWG) ($P < 0.001$) and significantly higher in the HNa group (1.86 kg·kg$^{-1}$ BWG) ($P < 0.001$) compared to control (1.77 kg·kg$^{-1}$ BWG) (Fig. 2). Apparently, the higher weight gain in the HS group was caused by better feed efficiency, whereas in the HNa group by higher feed intake.

Similar to our study, the best stimulating effect on the growth of chickens in the last weeks of experiment was observed in the study by Kocabağlı et al. (2002) who examined the effects of dietary humate (Farmagülaltör DRY$^{TM}$) supplementation at 2.5 kg/per ton of feed on broiler performance from day 0 to day 42. Body weights of chickens at 21 days were not affected by the dietary regimens. Body weights and feed conversion ratio of broilers were significantly affected by the dietary humate treatments at 42 days.
Significantly higher growth rate and better feed conversion were also observed in chickens fed diets containing 0.25% and 0.125% of humic substances (Farmagülatör DRY™) compared to chickens fed a diet without any supplements and chickens fed a diet containing natural mineral clay (Diatomaceous Earth) in the study of El-Husseiny et al. (2008).

Karaoglu et al. (2004) reported that humate supplementation to diets of broilers (at concentrations of 0.1, 0.2, and 0.3%) had no effect on the performance. A slight improvement (approximately 2% compared to control) was observed in the feed conversion ratio for the group fed the diet containing 0.1% humate.

Ozturk et al. (2010) found improved body weight gains and feed efficiency without affecting the feed intake of broiler chicken when humic acid was added to the drinking water at a dose of 300 ppm. The high humic acid-treated chickens (450 ppm) showed a reduction in body weight.

Yörük et al. (2004) observed that feed conversion efficiency (weight of feed/weight of eggs) in laying hens decreased linearly with increasing concentrations of supplemental humate.

Mortality in the control group fed the diet without supplementation was higher than that in groups fed diets containing humic substances (C 4.1%; HS 4.0%; HNa 0%). No changes in the health status of chickens were noticed during the whole experimental period. The cause of death of chickens in control and HS groups was the sudden death syndrome. Our results were similar to the results of studies by Yörük et al. (2004), Karaoglu et al. (2004) and Islam et al. (2008).

The values of European Efficiency Index were lower in both tested groups (321.9 for HS; 317.5 for HNa) compared to the control group on day 35 of the study (326.7) (Fig. 3) with a significant difference between the HNa and control groups ($P < 0.05$). The highest EEI values were observed in the HS group (342.7) ($P < 0.001$) on day 42 of the experiment due to more intense growth and better FCR, which represents a difference of 7.2% (319.7) and 8.0% (317.4) compared to control and HNa groups, respectively. The lowest EEI value was in the HNa group. A higher EEI in the treatment group compared to the control group was reported by Herzig et al. (2001) who...
spread a humin acid-based sorbent (oxyhumolite) onto the floor of the room where the experimental group of broiler chickens was kept.

The metabolic variables in blood serum analysed on days 14 and 35 of the study are shown in Table 4. While after 14 days of experiment, the concentrations of total protein and albumin were higher in both tested groups than in control, after 35 days of experiment the values of both variables were lower in the tested groups than in control. Differences between groups were not significant. The variables of energy metabolism after 14 days of the study showed non-significantly higher concentrations of glucose and total lipids, and lower concentrations of cholesterol and triglycerides in the HS group than in control. Higher concentrations of glucose, total lipids and triglycerides and significantly lower concentrations of cholesterol \( (P < 0.05) \) were observed in the HNa group compared to control. Values of the above mentioned energy metabolism variables were higher in the HS group than in control after 35 days. No significant differences were observed between groups. Lower concentrations of triglycerides, higher concentrations of cholesterol and total lipids, and significantly higher concentrations of glucose \( (P < 0.01) \) were measured in the HNa group compared to control after 35 days. The activity of AST after days 14 and 35 was lower in both treatment groups than in control with a significant difference after day 35 between control and the HS group \( (P < 0.05) \). Significantly higher activity of ALP in the HS group was observed in the HNa group compared to control \( (P < 0.001) \).
group than in the HNa group \((P < 0.05)\) was noticed after day 14. The difference between HS and control groups was not significant. Higher values of ALP activity in the HS group compared to the HNa and control groups but without any significant differences were also found after day 35. The increase of ALP activity may be induced by osteoblast activity, such as skeletal growth, which is greater in young and growing animals (Hassanabadi et al. 2007). Herzig et al. (2009) found non-significantly higher ALP activity in chickens after treatment with a diet containing humic acid (500 mg per chicken and day). Whereas in both tested groups higher contents of Ca and lower contents of P compared to control were found after day 14, lower contents of Ca and higher contents of P were found in tested groups compared to control after day 35. Significant differences were noticed in the contents of Ca between HS and control groups after day 14 \((P < 0.05)\) and between HNa and control groups after day 35 \((P < 0.05)\). The increase of serum Ca after day 14 can be one of the contributory factors of better growth and performance (Kadam et al. 2009). The reduction of serum concentrations of Ca after day 35 may be due to metal chelating effects of humic acids that are affected by a large number of carboxylic acid side chains (Klocking 1994).

Rath et al. (2006) using humic acids in the diets for broiler chickens at concentrations of 0.5, 1.0 and 2.5% observed their effect on serum chemistry values at high concentration after 5 weeks of trial. Except for cholesterol, triglyceride, creatinine and lactate dehydrogenase, there was a trend of decrease in protein, albumin, glucose, creatine kinase, blood urea nitrogen, alkaline phosphatase, alanine aminotranspherase, Ca, Fe and P concentrations.

Avei et al. (2007) reported that biochemical indicators such as P, K, Fe, Cu, Zn, total protein, glucose, cholesterol and triglyceride of Japanese quails were not affected by the dietary humic acids at amounts of 360, 480 and 600 mg/kg of diet. But concentrations of Ca were significantly increased in the experimental groups compared to control.

Significantly lower concentrations of cholesterol in serum of chickens fed a diet supplemented with humate (Farmagülätör DRY™) at concentrations 0.25 and 0.125% on day 35 of trial were found by El-Husseiny et al. (2008).

Kaya and Tuncer (2009) found that live weights, weight gains, feed intake, feed conversion ratio as well as serum total protein, triglyceride and cholesterol values in broiler chickens were not significantly affected by the addition of humic acids at the amount of 2.5 kg per ton of feed.

Comparing results of studies by many researches worldwide, performance differences due to humate supplementation might result from the compositional differences among the commercially available humate products (Kocabağli et al. 2002).

In conclusion, our study showed that the addition of natural humic substances to the broiler chicken diets yields better results on the performance than the addition of sodium humate. The final body weight was non-significantly higher in both tested groups than in the control group. The feed conversion ratio was significantly better in the HS group and worse in the HNa group compared to the control group. At the end of the experiment, on day 42 of chickens age, significantly higher EEI values were observed in the HS group due to more intensive growth and better feed conversion compared to the control and HNa groups.

Vplyv suplementácie kŕmnych zmesí prírodnými humínovými látkami a humátom sodným na produkciu a niektoré ukazovatele intermediárneho metabolizmu u brojlerových kurčiat

V pokuse bol sledovaný vplyv huminových látk z rozličných zdrojov na produkciu a niektoré biochemické ukazovatele u brojlerových kurčiat (n = 150, Ross 308) rozdeelených do kontrolnej (skupina C) a dvoch pokusných skupín (n = 50) kŕmených diéťou supplementovanou prírodnými humínovými látkami (skupina HS) a humátom sodným (skupi-
na HNa) v množstve 5 g·kg⁻¹ krmiva v prvej a 7 g·kg⁻¹ krmiva v druhej a tretej fáze výkrmu.
V oboch pokusných skupinách bola zaznamenaná nesignifikantne vyššia finálna hmotnosť kurčiat (HS 2527,6 g; HNa 2481,5 g) ako v kontrolnej skupine (C 2476,6 g). Za celé sledované obdobie bola v skupine HS zaznamenaná nižšia (P < 0,001) a v skupine HNa vyššia (P < 0,001) konverzia krmiva v porovnaní s kontrolnou skupinou. V skupine HS bola na konci pokusu stanovená vyššia (P < 0,001) a v skupine HNa nižšia hodnota Indexu efektivnosti výkrmu oproti kontrolnej skupine. Rozdiely v koncentrácií celkových proteinov, albuminu, celkových lipidov, triglyceridov a fosforu v krvnom sére medzi skupinami neboli štatisticky významné. Po 14 dňoch pokusu bola v skupine HS nameraná vyššia (P < 0,001), v skupine HNa nižšia (P < 0,001) konverzia krmiva v porovnaní s kontrolnou skupinou. V skupine HS bola na konci pokusu stanovená vyššia (P < 0,001) a v skupine HNa nižšia hodnota AST (P < 0,05) a v skupine HNa nižšia hladina glukózy (P < 0,01) ako v kontrolnej skupine.

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References