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Original article

Influence of humic substances on growth performance and blood serum parameters in fattening turkeys

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Abstract

The present study was conducted to evaluate the effect of humic substances on performance and selected blood biochemical parameters in turkeys. A total of twenty 6-week-old turkey hybrids (Big 6) were divided into two groups. The first group of turkeys was fed the basal diet without any supplementation of humic substances as a control group. The second group was fed the basal diet supplemented with 5 g of humic substances per kg of diet. The study lasted 35 days, until the 11th week of age of the turkeys. The addition of humic substances had no effect on the live body weight, body weight gain, feed intake, feed conversion ratio, or growth rate of turkeys. Similarly, the monitored blood biochemical parameters, except for the levels of P, Mg, and Na, were not significantly affected by the addition of humic substances. The addition of humic substances led to a significant decrease in the concentration of P ($p<0.05$), Mg ($p<0.05$), and Na ($p<0.01$) in the blood serum of turkeys. It was concluded that the addition of humic substances may lead to a reduction of some mineral substances in the blood of animals, which may be due to their chelating effects. This may indicate a reduced availability of some biologically important minerals for the body, which could subsequently manifest itself in some health issues and a decrease in animal production. During the duration of our study, there were no signs of disturbance in the health of the animals. In view of the achieved results, further studies will be necessary to determine the appropriate concentration and duration of administration of the monitored preparation and to evaluate its influence on the availability of nutrients and feed conversion and its possible use as a safe non-antibiotic growth stimulator in turkey nutrition.

Keywords: blood metabolites, growth performance, natural feed additives, poultry

Introduction

In connection with the ban on the use of antibiotic growth stimulants, innovations in animal feeding are leading to the use of various groups of additives of natural origin. These are substances that lead to improved feed conversion, maintain animal health, reduce costs, and bring economic benefits without negative effects on animal or human health. Humic substances are one of the many alternatives to antibiotic growth stimulants. These organic compounds result from the decomposition of plant and animal remains. They occur naturally in rock sediments, peat, brown coal, lignite, and some other materials. They are the main component of soil humus. They contain humic, fulvic, and ulmic acids, humin, and trace minerals, which are essential for plant growth (Stevenson 1994, Bezuglova and Klimenko 2022).

The application of preparations based on humic substances is widespread both in plant production with the aim of stimulating plant growth (Bezuglova and Klimenko 2022) and in medicine, where they are used to treat various diseases (poisoning with heavy metals, diseases of the gastrointestinal tract, etc.). Antidiarrheal, analgesic, anti-inflammatory, and antimicrobial effects are attributed to humic substances (Kucukersan et al. 2005, Sahin et al. 2016). They are also characterized by their ability to bind heavy metals and organic and inorganic compounds, including toxic pollutants such as pesticides and other environmental pollutants, forming chelates (Sahin et al. 2016, Bezugluva and Klimenko 2022). By binding toxic substances, they positively affect soil hygiene and prevent their transfer to agricultural crops (Veselá et al. 2000, Kucukersan et al. 2005). Likewise, in the case of body intoxication, the application of humic substances can be very useful since, by binding toxic substances, they prevent their action in the body and enable their elimination from the body (Vašková et al. 2023).

The possibility of using humic substances in animal production has recently received considerable attention. There are a number of scientific publications on the benefits of using humic substances as feed additives. After their application in feed or water, they have a positive effect on animal growth, feed conversion (Taklimi et al. 2012, Abdel-Mageed 2012, Mirnawati and Marlida 2013, Arif et al. 2016, Hammod et al. 2021), and an increase in slaughter yield (Abdel-Mageed 2012, Marcinčáková et al. 2015). An improvement in egg production was noted in laying hens (Kucukersan et al. 2005, Mudroňová et al. 2021). They can also contribute to the reduction of animal mortality rates (Abdel-Mageed 2012). Positive effects were also demonstrated on animal immunity (Mudroňová et al. 2020, Mudroňová et al. 2021, Bujňák et al. 2023), blood cholesterol level (Ozturk et al. 2012, Jaďuttová et al. 2019), and on the quality of poultry meat (Semjon et al. 2020, Gálik et al. 2023). The advantages of these natural substances are their easy application to diets and drinking water and the absence of residues in animal products (Trckova et al. 2005). The positive effect of humic substances on the production indicators of animals is attributed to their ability to increase the utilization of feed nutrients, for example, by increasing the height of the villi of the intestinal mucosa, which leads to an increase in the absorption surface (Abdel-Mageed 2012, Taklimi et al. 2012), and by increasing the digestibility of nutrients through a positive effect on the enzymatic activity in the digestive tract (Marcin et al. 2023) or through the stabilization of intestinal microflora. They increase the population of beneficial bacteria, and lead to a reduction in the number of pathogens in the digestive tract (Mudroňová et al. 2020, Agboola et al. 2021, Omidiwura et al. 2022).

Humic substances can also contribute to the improvement of air quality in stable premises by reducing the concentration of ammonia, which is produced by the microbial activity of nitrogen excreted by excrements in the litter. This effect can consist in the application of humic substances to the bedding (Suchy et al. 1999, Herzig et al. 2001) or in an increase in the use of proteins from the feed and subsequently in the reduction of nitrogen excretion due to the feeding of humic substances (Hreško Šamudovská et al. 2022).

Although there is quite a lot of knowledge about the influence of humic substances on the production and health of poultry, especially broiler chickens, laying hens, and quails, the results of various studies differ. There is also little information about the possibilities of using these natural substances in the nutrition of turkeys, whose breeding is an important branch of poultry breeding, and the demand for its products is growing worldwide. They are bred for tasty and high-quality meat, which is characterized by low fat and cholesterol content, high protein content, and delicate flavor (Agina et al. 2015, Shah et al. 2023).

The aim of this research was to determine the effect of humic substances on the production and blood parameters of turkeys from 6 to 11 weeks of age under experimental conditions.

Materials and Methods

Animals and experimental design

Twenty 6-week-old turkeys of the Big 6 hybrid were included in the experiment, and after being weighed and marked with an identification ring, they

Table 1. Analyzed content of nutrients in feed mixtures for turkeys.

		Control group	Experimental group
Dry matter	(g/kg)	882.40	878.90
Crude protein	(g/kg)	210.75	206.58
Ether extract	(g/kg)	34.83	34.81
Crude fibre	(g/kg)	44.78	44.63
Starch	(g/kg)	416.62	413.07
Ash	(g/kg)	69.25	72.30
Calcium	(g/kg)	10.05	10.51
Sodium	(g/kg)	1.59	1.59
Phosphorus	(g/kg)	6.07	6.05
Copper	(mg/kg)	20.40	20.43
Zinc	(mg/kg)	125.87	128.43
Metabolisable energy	(MJ/kg)	12.06	11.94

were divided into two groups (control and experimental; n=10). The average weight of the turkeys at the beginning of the experiment was 1737±59.29 g. They were housed on deep bedding under standard environmental conditions (with controlled temperature and humidity). The stocking density in the pen was according to valid standards for turkey breeding. During the experiment, the turkeys of both groups were fed a complete feed mixture based on corn, soybean meal, peas, wheat, rapeseed meal, and barley (feed mixture for fattening turkeys from 5 to 12 weeks of age, Midi forte turkey; DeHeus s.r.o. Kendice, Slovak republic). In the experimental group, humic substances were added to the feed mixture in the amount of 5 g/kg (HUMAC MycotoxiSorb; Humac s.r.o., Košice, Slovakia): particle size up to 200 μm, pH 5.8; humidity max. 21%; humic acids min. 65%; and fulvic acids 5% in dry matter. Feed intake and water were provided ad libitum throughout the experiment. The chemical analysis of feed mixtures was carried out by analytical methods according to Commission Regulation (EC) No. 152/2009. The analyzed content of nutrients in the feed mixtures is presented in Table 1. During the entire monitored period (35 days), the weight of the turkeys and feed consumption were recorded regularly. Growth rate was calculated according to the formula: [(final weight minus initial weight) / (0.5 x (initial weight plus final weight))] x 100. Feed conversion was expressed as feed consumption per kilogram of gain.

This experiment was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice (protocol no. 2022/11).

Laboratory analysis

Blood samples for serum biochemical examination were collected on the 35th day of the experiment, at the age of 11 weeks, from 8 turkeys in each group from the ulnaris vein. Biochemical parameters in serum (total proteins, albumin, uric acid, glucose, triacylglycerols, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyl transferase (GGT), alkaline phosphatase (ALP), calcium, phosphorus, magnesium, sodium, potassium, chlorides, iron, copper, and zinc) were measured using an ADVIA® 1800 automatic analyzer (Siemens). Globulin concentration was calculated by subtracting albumin values from total protein values. The ratio of albumin to globulin was calculated using the formula: A/G ratio = Albumin / (Total protein minus Albumin). The concentration of malondialdehyde (MDA) in the blood serum, as an indicator of the degree of lipid peroxidation and oxidative stress, was determined by the spectrophotometric method of determining the content of substances reactive with thiobarbituric acid (TBARs, thiobarbituric acid reactive substances), according to Costa et al. (2006). Results are expressed as nmol MDA/ml serum. A decrease in oxidative stress is characterized by a decrease in MDA levels (TBARs).

Statistical analysis

The statistical evaluation of the effects of humic substances on monitored parameters was done by unpaired *T*-test with GraphPad Prism 8.0 statistical software (GraphPad Software, San Diego, California, USA). Results were expressed as the means and the pooled standard error of the mean (SEM). The values of $p<0.05$ were set as statistically significant.

Results

Production parameters

The average live weight of turkeys, weight gain, feed intake, feed conversion, and growth rate were not

Table 2. Effect of supplementation of feed mixtures with humic substances on selected production parameters in fattening turkeys aged 6–11 weeks.

		CG	EG	SEM	p-Value
Average body weight (11th week)	(g)	5945.71	6221.43	133.481	0.321
Average daily weight gain	(g)	121.74	126.64	3.057	0.445
Average daily feed intake	(g)	278.13	280.90	32.013	0.971
Feed conversion ratio	(kg/kg)	2.29	2.24	0.054	0.656
Growth rate		111.74	110.80	1.803	0.806

CG – Control group, EG – Experimental group, SEM – Pooled standard error of the mean

statistically significantly affected by the addition of humic substances to the feed mixture in the amount of 5 g/kg in the period from 6 to 11 weeks of age (Table 2). However, the experimental group recorded a numerically higher average live weight of turkeys, higher average daily weight gain and better feed conversion ratio for the entire monitored period compared to the control group. The difference in the average live weight of turkeys was 4.64 %, the difference in weight gain was 4.02% and the difference in feed conversion ratio was 2.23%.

Serum parameters

The values of the monitored biochemical parameters in the blood are shown in Table 3. The addition of humic substances did not significantly affect the level of monitored indicators of protein (total protein, albumin, globulin, uric acid) and energy metabolism (glucose, triacylglycerols, cholesterol), lipid peroxidation indicators (TBARs), nor the activity of liver enzymes (AST, ALT, GGT, ALP) in the blood of turkeys. However, it led to a significant decrease in the levels of phosphorus ($p<0.05$), magnesium ($p<0.05$), and sodium ($p<0.01$). The concentrations of calcium, potassium, chlorides, iron, copper, and zinc in the blood of turkeys were not statistically significantly affected by the addition of the monitored product to the feed. However, in the experimental group, there was a trend toward decreasing the concentration of triacylglycerols ($p<0.10$) and potassium ($p<0.10$) compared to the control group.

Discussion

In various studies, it has been found that the addition of humic substances to feed or drinking water can lead to an improvement in the production parameters of poultry. For example, Taklimi et al. (2012), who observed the effect of the addition of humic acids in different concentrations to feed mixtures (0.2 and 0.3%) in broiler chickens, noted a significant increase in weight gain as well as a significant improvement in feed conversion. These results are in agreement with those reported by Arif et al. (2016), ELnaggar and El-Kelawy (2018), and Hammod et al. (2021) in chickens fed a feed mixture with the addition of humic acids, as well as Mirnawati and Marlida (2013) in chickens to which humic acids were added to water. We also noted a significant improvement in feed conversion in broiler chickens due to the addition of natural humic substances to the feed (in the amount of 5 g/kg in the first and 7 g/kg in the second and third fattening phases) in our earlier study (Šamudovská and Demeterová 2010).

Similar results were also recorded by Abdel-Mageed (2012) in Japanese quail. Quails that received feed with the addition of humic substances (10, 20, or 30 ml of humic substances per kg of feed) achieved significantly higher final weight, significantly higher weight gains, significantly better feed conversion, and significantly higher growth rate throughout the observed period compared to the control group.

However, in our experiment, the monitored production parameters in turkeys (live weight, weight gain, feed conversion, and growth rate) were not affected by the addition of humic substances to the feed. Similar results were recorded in their studies by Kaya and Tuncer (2009), Jaďuttová et al. (2019), Domínguez-Negrete et al. (2019) and Hudák et al. (2021) in broiler chickens.

Quantitative determination of biochemical parameters in blood serum helps to assess the effect of the monitored preparation on the absorption of nutrients, on the metabolism of basic biochemical compounds, on the functional state of vital organs of the body, and to assess the toxicity of this preparation. Compared to the control group, a significantly lower level of phosphorus ($p<0.05$), magnesium ($p<0.05$), and sodium ($p<0.01$) was recorded in the blood serum of the turkeys in the experimental group, as well as a trend of decreasing potassium concentration ($p<0.10$). A significant decrease in the concentration of phosphorus in the blood of poultry due to the addition of humic substances to the feed was also noted by Celik et al. (2008) and Abdel-Mageed (2012). Abdel-Mageed (2012), in addition to a significant decrease in phosphorus, also noted

Table 3. Effect of supplementation of feed mixtures with humic substances on selected blood serum parameters in fattening turkeys at the age of 11 weeks (35th day of administration of humic substances).

		CG	EG	SEM	p-Value
Indicators of protein metabolism:					
Total protein	(g/l)	39.13	38.97	0.810	0.929
Albumin	(g/l)	19.35	19.41	0.356	0.931
Globulin	(g/l)	19.78	19.56	0.510	0.841
A/G ratio		0.98	1.00	0.016	0.497
Uric acid	(µmol/l)	389.97	332.76	18.672	0.129
Indicators of energy metabolism:					
Glucose	(mmol/l)	15.90	16.02	0.143	0.693
Triacylglycerols	(mmol/l)	1.35	0.99	0.100	0.071
Cholesterol	(mmol/l)	3.85	3.65	0.119	0.412
Activity of liver enzymes:					
AST	(µkat/l)	5.15	5.09	0.102	0.770
ALT	(µkat/l)	0.03	0.03	0.003	0.693
GGT	(µkat/l)	0.06	0.05	0.005	0.414
ALP	(µkat/l)	29.62	28.80	1.180	0.741
Indicators of mineral metabolism:					
Calcium	(mmol/l)	3.03	2.99	0.044	0.676
Phosphorus	(mmol/l)	2.70[a]	2.47[b]	0.050	0.019
Magnesium	(mmol/l)	0.98[a]	0.88[b]	0.020	0.018
Sodium	(mmol/l)	157.00[a]	154.63[c]	0.467	0.006
Potassium	(mmol/l)	3.63	3.20	0.127	0.096
Chlorides	(mmol/l)	116.00	114.63	0.435	0.117
Iron	(µmol/l)	33.80	33.19	1.298	0.823
Copper	(µmol/l)	1.54	1.84	0.129	0.248
Zinc	(µmol/l)	43.48	42.07	1.237	0.586
Indicator of lipid peroxidation and oxidative stress:					
TBARs	(nmol MDA/ml)	0.67	0.69	0.021	0.626

CG – Control group, EG – Experimental group, SEM – Pooled standard error of the mean, A/G ratio – Albumin/Globulin ratio, AST – Aspartate aminotransferase, ALT – Alanine aminotransferase, GGT – Gammaglutamyl transferase, ALP – Alkaline phosphatase, TBARs – thiobarbituric acid reactive substances.
Values marked with a different superscript in the same row are statistically significantly different ([ab] $p<0.05$, [ac] $p<0.01$).

a significant decrease in calcium. These results are in agreement with the results reported in their study by Nad' et al. (2021). In the blood of broiler chickens that received feed with the addition of humic substances (at a concentration of 0.7%), a significantly lower concentration of calcium, phosphorus, and chlorides was measured compared to the control group. A significant sodium reduction, similar to our study, was reported by Kovacik et al. (2020) in pheasants that were given feed with the addition of humic acids in a 1% concentration.

It is assumed that the decrease in the concentration of some mineral substances in the blood of animals receiving humic substances may be due to their possible chelating effects due to their high molecular weight and the number of functional groups (Ozturk et al. 2014, Sahin et al. 2016). The carboxyl groups of humic acids are among the main contributors to their chelating properties (Vašková et al. 2023). The formation of chelates with undesirable substances, such as heavy metals, leads to a reduction in their adverse effect on the organism and enables their elimination from the body (Sahin et al. 2016, Vašková et al. 2023). However, it is questionable whether the chelation of biologically important elements available in the feed could lead to their reduced availability for the organism. Lack of biologically important elements can affect growth,

bone mineralization, muscle function, enzyme activity, normal metabolism, and, in laying hens, egg production and eggshell quality (Scanes and Christensen 2020). In our study, we recorded a significant reduction of some mineral substances in the blood serum of turkeys after the addition of humic substances to the feed, but these measured values did not significantly differ from the values reported in their study by Szabo et al. (2005).

These results are in contrast to those reported by Lala et al. (2016). Significantly higher concentrations of phosphorus and calcium were found in the blood of broiler chickens that received humic acids through drinking water (1 and 2 ml/l) compared to the control group. A significant increase in calcium concentration due to humic acids (600 mg/kg feed) was also recorded in Japanese quail (Avci et al. 2007). Other monitored parameters of the mineral profile (P, K, Fe, Cu, and Zn) as well as the parameters of the protein (total protein) and energy (glucose, triacylglycerols, and cholesterol) profiles in the blood serum of the quails were not significantly affected.

Likewise, in our study, blood biochemical parameters, such as total proteins, glucose, triacylglycerols and cholesterol, and also albumin, globulin, A/G ratio, and uric acid, were not statistically significantly affected by the addition of humic substances to turkey feed. However, in the blood of turkeys in the experimental group, compared to the control group, a trend of decreasing triacylglycerol concentration was recorded ($p<0.10$).

A decrease in the concentration of triacylglycerols in the blood serum due to the addition of humic substances to the feed was also found in chickens (ELnaggar and El-Kelawy 2018). In addition, significant decreases in cholesterol, total lipids, albumin, and A/G ratio and significant increases in glucose, total proteins and globulin were also noted.

In the study, Ozturk et al. (2012) also observed the influence of cholesterol concentration in the blood of chickens by supplementing humic substances in the feed. Significantly lower cholesterol levels were measured in the chickens that were fed with the supplemented feed (the addition of humic substances in amounts of 1 and 1.5 g/kg) than in the control group. The concentrations of glucose, protein, and triacylglycerides in the blood of chickens were not significantly affected.

The reduction of the levels of triacylglycerols and cholesterol in the blood of animals due to the addition of humic substances indicates that these natural substances could also be used in the prevention and treatment of cardiovascular diseases.

Some studies suggest that humic substances may have antioxidant properties and may protect against diseases related to oxidative stress. These significant antioxidant properties of humic substances were confirmed in Mao's study (2019). A significantly higher activity of enzymes was involved in the antioxidant defense system (superoxide dismutase and glutathione peroxidase) and there was a lower MDA level compared with a control group. Similarly, a decrease in MDA levels in blood serum was also noted by Kovacik et al. (2020) in pheasants that were fed feed with the addition of humic acids in 0.5, 0.75, and 1.0 % concentrations. The MDA values decreased significantly with the increasing level of humic acids in the feed.

However, in our study, only slight and insignificant changes in the level of MDA (TBARs) in the blood serum of turkeys due to the addition of humic substances to the feed were observed. Similar results were reported by Zhang et al. (2020) in laying hens. Ipek et al. (2008), who observed the effect of humic acids in Japanese quail, found that a high content of humic acids in the feed (600 mg/kg) could lead to an increase in oxidative stress. While high concentrations of humic acids led to a decrease in total antioxidant capacity, their lower concentrations (360 or 480 mg/kg) had no effect.

By analyzing the activity of liver enzymes in the blood serum of turkeys, it was demonstrated that the addition of humic substances to the feed in the amount of 5 g/kg has no harmful effect on liver function. The values of AST, ALT, GGT, and ALP in the experimental group were comparable to the values in the control group. During the duration of the experiment, no deaths were recorded in the control or experimental groups of animals, and no signs of health impairment were recorded.

The results of various studies show that the influence of humic substances on poultry production as well as on biochemical parameters in the blood can be different. This inconsistency in the results obtained in different studies can be attributed to the composition and amount of humic substances administered, the method of their application (in feed or in water), and the type of animals used. This fact indicates the need to continue research on humic substances focused on animal health and the possibility of their use in animal nutrition.

However, the effect of the available preparations of humic substances, due to their different compositions (mainly the representation of the main components, the ratio of humic and fulvic acids), needs to be assessed individually.

In conclusion, the addition of humic substances to the feed in the amount of 5 g/kg did not have a significant effect on the observed production parameters, such

as live weight of turkeys, weight gains, feed intake, feed conversion, and growth rate. However, it led to a significant reduction of phosphorus, magnesium, and sodium in the blood serum of turkeys. In view of the achieved results, further studies will be necessary to determine the appropriate concentration and duration of administration of the monitored preparation and to evaluate its influence on the availability of nutrients and feed conversion and its possible use as a safe non-antibiotic growth stimulator in turkey nutrition.

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