The Effect of Humic Substances on the Content of Copper and Zinc in the Turkey Muscle

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ABSTRACT— ***The aim of the study was to assess the effect of humic substances on the changes of copper (Cu) and zinc (Zn) content in the thigh and breast muscle of turkey. For the trial, 20 turkeys (BIG 6) were allocated into two groups (control group and experimental group) of 10 birds. Experimental group was supplemented with 0.5 % Humac Mycotoxi Sorb (HM). The control group received a basal diet without any supplements. The Humac Mycotoxi Sorb was added to turkey feed for 10 weeks. In the breast and thigh muscles, we found a statistically significantly higher (P < 0.001) Cu content after the addition of 0.5% HM to the feed (4.43; 4.18 mg.kg^{-1}) compared to the control group of turkeys (2.70;3.35 mg.kg^{-1}). Also, in the breast and thigh muscles, were found a statistically significantly higher (P < 0.05) Zn content after the addition of 0.5% HM to the feed (50.98; 64.42 mg.kg^{-1}) compared to the control group of turkeys (44.33; 62.95 mg.kg^{-1}).***

Keywords— Humic substances, Turkeys, Muscle, Copper, Zinc

1. INTRODUCTION

The influence of black coal on the digestive tract was already known to people in the Middle Ages, but the fact that humic acids play a role in these processes has only been known for a relatively short time. Research in this area has seen progress only in the last decade, which conditioned the increasingly frequent use of preparations based on humic acids not only in human medicine, but also in plant and animal production. Humates as feed additives are substances that are intentionally added to feed or water to fulfill their function. Their role is to have a beneficial effect on the properties of products of animal origin, to satisfy the needs of animals regarding nutrition, to have a beneficial effect on the environment [11].

Humates include humus, humic acid, fulvic acid, ulmic acid, and trace minerals. Their effectiveness is based mainly on the effect on the microflora of the digestive tract or feed digestion [19]. Humic substances very strongly bind toxic metals in chelate bonds and thus reduce their undesirable effects in the soil and in the digestive tract [18]. The absorptive capacity and chelation of metals by humic substances can reduce the absorption of biologically important elements that are available to the animal [20]. Differentiated effects were shown by humic acids for trace elements, especially for copper and zinc [5]. Feeding humic substances increased the levels of some essential minerals (such as Ca, Al and Fe) in the serum, liver and muscle of poultry [16].

The aim of the work was to monitor the influence of humic substances on the content of copper and zinc in the thigh and breast muscles of turkeys.

2. MATERIALS AND METHODS

2.1 Animals

In the experiment, 42-day-old turkeys of the BIG 6 type were used. The turkeys were placed on deep bedding to ensure and control the microclimate conditions. They were divided into two groups, control and experimental. The average weight of turkeys at the age of 6 weeks, at the beginning of the experiment, was 1 735 g. The turkeys were fed twice a day with industrially produced feed mixtures Turkey Midi - diet for turkeys from 9-12 weeks of age, Turkey Maxi - diet for turkeys from 13 weeks of age until slaughter (DE HEUS s.r.o. Kendice, Slovak Republic), during the entire period trial duration, i.e. 10 weeks.

2.2 Experimental desing and diet

Turkeys from the control group were fed feed without addition of humic substances. From the 6th week of age, the turkeys of the experimental group were given HUMAC Mycotoxi Sorb (HM) in a concentration of 0.5% in the form of a fine gray-black powder. Turkeys had access to feed and water *ad libitum*. During the experiment and at the end of the experiment, the turkeys were weighed. They were killed in accordance with the code of ethics, by cervical dislocation and slaughtered.

2.3 Muslce sample

Breast and thigh muscle samples were processed with a Milestone mineralization system (MLS 1200 Mega) with microwave decomposition technology. The analysis of the samples for the presence of copper and zinc was carried out on Atomic absorption sprectrometer (Unicam Solar 939, UK) by the flame method. The methodology specified in the List of official methods and laboratory diagnostics of food and feed (Vestník MPSR, 2004) was used for the determination. The values presented in the tables (table no. 2 and 3) are average values from 8 samples of breast and thigh muscle from each group.

4.2 Ethical statement

The protocol for animal fatterning was approved by the Ethics Committee for Anima Care and Use of the University of Veterinary Medicine and Pharmacy in Košice (with the consent of the State Veterinary and Food Administration of the Slovak Republic under number EKVP/2022-11). The experiment was carried out on the premises for poultry housing of the University of Veterinary Medicine and Pharmacy in Košice (Slovak republic).

5.2 Statistical analysis

The data obtained from this experiment were evaluated using GraphPad Prism 3.0 and expresed as mean ±standard deviation (X±SD). Tukey comparison test was used to compare the differences between the values of the monitored grops and $P \leq 0.05$; $P \leq 0.001$ was considered a significant difference.

3. RESULTS

The addition of humic substances in feed ensures a good health condition of animals and has a positive effect on production parameters and does not require protection periods. Table No. 1 shows the average weekly gains and the average live weight of the turkeys during the experiment. On the 6th week of the experiment, the average live weight of turkeys in the control group was 6 995 g and in the group of turkeys fed 0.5% HM 7 107.50 g. At the end of the experiment, week 10, the average live weight of turkeys in the control group was 9 985 g and the average live weight of turkeys fed 0.5% HM was 10 505 g. Compared to the control group, it was 520 g higher. Feeding 0.5% HM caused better average daily gains of turkeys (125.94 g) during the duration of the experiment compared to the group of turkeys that were fed the feed mixture without the addition and the average daily gains were lower (117.43 g).

Table 1: Average body weight and average weekly gains of turkeys (in grams)

Groups	**Control**		**0.5 % HM**	
	weight	weight gain	weight	weight gain
2nd week	3 136.00	100.69	3 244.00	107.26
4nd week	4 953.75	123.64	5 005.00	132.77
6nd week	6 995.00	145.80	7 107.50	150.18
8nd week	8 488.75	106.70	8 956.25	132.05
10nd week	9 985.00	106.88	10 505.00	110.63

In tables No. 2 and 3 show the average contents of copper and zinc in breast and thigh muscles of turkeys. In the control group of turkeys, the average levels of Cu in breast and thigh muscles were almost the same (2.70; 3.35 $mg.kg^{-1}$). The values found by us did not exceed the maximum permitted occurrence limit for honey in muscle tissue (5.0 $mg.kg^{-1}$) compared to the Food Code of the Slovak Republic (EC No. 629/2008).

In the muscle of turkeys from the group fed with the addition of 0.5% HM, the average Cu values were higher (4.43; 4.18 $mg.kg^{-1}$). Addition of 0.5% HM ($P \leq 0.001$) to the feed significantly increased the amount of Cu in the breast

muscle compared to the control group, similarly in the thigh muscle there was a significant increase ($P \leq 0.05$) in the copper content compared to the control group.

The maximum levels of copper in two turkey breast muscle samples (5.10; 5.50 $mg.kg^{-1}$) and two turkey thigh muscle samples (6.00; 5.90 $mg.kg^{-1}$) in the group with the addition of 0.5 % HM in the feed, which exceeded the maximum permitted occurrence limit for honey in muscle tissue (5.0 $mg.kg^{-1}$) compared to the Food Code of the Slovak Republic (EC No. 629/2008).

Table 2: The contentt of copper in the muscles of turkeys

Group	Muscle	Cu $mg.kg^{-1}$
Control	Breast	2.70 ± 0.14
	Thigh	3.35 ± 0.59
0.5 % HM	Breast	4.43 ± 0.83***
	Thigh	4.18 ± 1.62*

The data are means of 8 samples of breast and thigh muscles from each group; * $P \leq 0.05$ ***$P \leq 0.001$

Table 3: The contentt of zinc in the muscles of turkeys

Group	Muscle	Zn $mg.kg^{-1}$
Control	Breast	44.33 ± 13.95
	Thigh	62.95 ± 6.87
0.5 % HM	Breast	50.98 ± 4.48 *
	Thigh	64.42 ± 12.83 *

The data are means of 8 samples of breast and thigh muscles from each group; * $P \leq 0.05$

4. DISCUSSION

Humic substance are considered to be a suitable alternative to antibiotic growth promoters in poultry diets. Nutritionists are constantly looking for viable additives because conventional supplements have been criticized for negatively affecting the food chain. The advantage of adding humic acids to the ration of broiler chickens is that the products of these animals are free of residues of foreign substances. In the group of broilers fed with feed mixture for broilers with the addition of 0.7% Humac Natur Monogastric modified with acid calcium formate, there were better live weight gains compared to the control group [15].

Marcinčáková et al. (2017) [9], recorded gains in broilers fed a feed mixture with the addition of 0.8% humic acids compared to the control group. Better growth parameters, greater feed efficiency and minor mortality have been recorded in broilers fed with the addition of this organic and nontoxic matter [6; 12]. In contrast, some studies showed a non-significant effect on the broilers during the fattening period [11].

Humic substances have shown strong affinity for binding various substances, such as heavy metals [7], minerals [2] and aflatoxins [14]. It has been indicated that HA had differentiated effects upon trace elements in rats. Plasma iron levels were hardly affected, while copper and zinc levels were initially suppressed with a tendency for recovery after 60 days. Feeding humic substances increased levels of some essential minerals (such as Ca, Mg and Fe) in serum, liver and poultry muscles [16].

The values found by us did not exceed the maximum permitted occurrence limit for honey in muscle tissue (5.0 $mg.kg^{-1}$) compared to the Food Code of the Slovak Republic (EC No. 629/2008). On the other hand, we found a significantly higher content of Zn in the muscles of the turkeys, and the maximum permitted limit for zinc in the muscles (50.0 $mg.kg^{-1}$) was exceeded compared to the Food Code of the Slovak Republic (EC No. 629/2008). Several authors report similar or lower levels of Cu in chicken muscle in Brazil of 0.3 - 3.5 $mg.kg^{-1}$ [3]; in Turkey, 0.5 to 12.3 $mg.kg^{-1}$ [17]; 0.27 - 0.82 $mg.kg^{-1}$ in China [4].

Similarly, the maximum permitted limit of zinc occurrence in muscle was also found in breast and thigh muscle samples of turkeys from the group with the addition of 0.5% HM to the feed.

In Pakistan, Khan et al. [8] found much higher values in thigh muscle (107.4 ± 7.60; 106.6 ± 7.37 and 106.78 ± 7.48 ppm) and breast muscle (107.82 ± 7.66; 107, 4 ± 7.49 and 107.95 ± 7.73) taken from different areas. Mariam et al. [10] found lower levels of Zn in poultry muscle (28.52 ppm). Žatko et al. [20] focused in their work on potential antagonistic relationships between iron and manganese, copper and zinc, etc. Antagonistic relationships between zinc and copper lie in their competition during intestinal absorption. Elemental levels detected by atomic absorption spectrometry indicated

particularly significant changes in the amounts of metal present. Cu was higher in liver and plasma but lower in kidney. The amount of Zn decreased in the liver and kidney, as did Mn.

5. CONCLUSION

In conclusion, we can conclude that feeding 0.5% Humac resulted in an increase in the content of monitored elements, especially zinc in the muscles of turkeys. Since humic substances are considered to be natural ligands with a high complexing ability, with an almost infinite number of metals, to reduce the occurrence of some risk elements in the muscles of animals. The presence of several metals inside cells and their interactions with biological ligands are factors that can change the biological effect of humic acids.

Based on the results, we can conclude that the use of humic substances as a supplement in poultry nutrition has its justification for maintaining the healthy growth of farm animals.

6. ACKNOWLEDGEMENT

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