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IMPACT OF DIETARY HUMIC SUBSTANCES SUPPLEMENTATION ON SELECTED MINERALS IN MUSCLES OF BROILER CHICKENS

Skalická, M., Naď, P., Bujňák, L., Marcin, A.
Department of Animal Nutrition and Husbandry
University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 04181 Košice
Slovakia

magdalena.skalicka@uvlf.sk

ABSTRACT

In this study, we assessed the effect of humic substances on the changes of some mineral (Ca, Mg, Cu and Zn) content in the thigh and breast muscle of broilers. Group 1 (G1) was supplemented with 0.7 % Humac Natur Mycosorb (HNMy), and G2 with 0.3 % HNMy. The control group (GC) received a basal diet without any supplements. In the breast muscle of broilers from the group G1 we found a statistically significantly higher ($P < 0.05$) Ca content and significantly reduced ($P < 0.05$) in the thigh muscle Ca content from the group G2 compared to the control group (GC). A statistically significant increase in Mg content ($P < 0.05$) was analyzed in breast muscles in the group G1 and also significant reduction ($P \leq 0.01$) in Cu in the breast and thigh muscles was found in the group G1 and in the breast muscle from the group G2 compared to the GC. Significantly lower content of Zn ($P \leq 0.05$) was found in the breast and thigh muscles of broilers in the group G2 and strong positive correlation ($r = 0.9093$) were observed between Ca in breast muscle from the group G2 and Ca in thigh muscle from the control group. A negative correlation ($r = -0.7656$) was shown for Mg between thigh muscle from the group G2 and Ca in thigh muscle from the GC. A strong negative correlation was confirmed ($r = -0.9221$) for Zn content in breast muscles between groups G2 and G1. The high positive correlation for Zn was occurred between breast muscle from the control group and thigh muscle from the group G1 ($r = 0.9786$).

Key words: AAS; broilers; calcium; copper; humic substances; magnesium; zinc

INTRODUCTION

The nutritional value and the outstanding taste of chicken meat have given rise to an increase in its consumption in a number of countries. Chicken meat is a popular ingredient in our diet, people consume it mainly for its organoleptic qualities (colour, taste), but also for nutritional reasons—full of proteins, vitamins and minerals. This means that the search for the new ways to improve the production yield of this animal species is of considerable interest. In recent years, there is growing interest in the use of humic compounds in animal feeding. The supplemental humic substances (HS) as a g warowth-promoting agent has multiple health effects and nutritional benefits for domestic animals [15]. The effect of humic compounds used in animal nutrition on rearing performance improved the results obtained for growth rate, feed utilization, meat quality, egg yield, and egg shell thickness and strength [1, 3, 10, 16, 26]. Some studies investigated the effect of using humic acids (HA) as growth promoter in poultry and obtained positive results [9,17]. Humic substances have shown strong affinity for binding various substances, such as heavy metals [11, 13], minerals [5] and aflatoxins [19, 25]. 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 [22]. There are interactions between the individual chemical elements [2], the mutual manifestation manifests itself synergistically or antagonistically, which takes place in feed, in the digestive tract, as well as in the process of tissue and cellular metabolism.

The aim of this study was to find out the influence of humic substances on the changes of mineral content (Ca, Mg, Cu and Zn) in the thigh and breast muscle of broiler chickens.

MATERIALS AND METHODS

One-day-old chickens of hybrid ROSS 308 were randomly divided into 3 groups (n = 30). The average body weight of the chickens was 37.30 g. **Dietary treatments were as follows:** the broilers were fed commercial feed mixture BR1, diet for fattening broilers within 10 days of age, BR2 diet for growing to 30 days of age and BR3 final feed mixture (AGROCASS plus, Ltd. Čaňa, Slovakia) for the duration of the experiment (42 days).

Ethical statement

The experiment was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice, the Slovak Republic. All procedures in this study were performed in accordance with the principles of the European Directive on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Parliament and Council, 2010).

Composition of BR1 diet for fattening

Maize 35.00 %; wheat 35.00 %; soybean meal 21.30 %; dried blood 1.25 %; limestone 1.00 %; monocalcium phosphate 1.00 %; salt 0.10 %; lysine 1.20 %; methionine 0.60 %; premix 0.50 %. Chemical composition: metabolic energy 12.01 IU; nitrogenous substances 22 %; ash 6 %; fat 2.5—5.0 %; crude fibre max. 4.00 %; nonphytate phosporus min. 0.42 %; Ca min 0.9 %; Na min 0.15 %; retinol 12 500 IU.kg^{-1}; Cholecalciferol 3000 IU.kg^{-1}; alfa-tocoferol 50 IU.kg^{-1}. Antioxidants: propylgallat 100 mg.kg^{-1}. Coccidiostats: narazin 70 mg.kg^{-1}.

Composition of BR2 diet for growing

Maize 40 %; wheat 35 %; soybean meal 18.70 %; limestone 1.05 %; monocalcium phosphate 0.70 %; salt 0.15 %; lysine 1.15 %; methionine 0.46 %; premix 0.50 %. Chemical composition: metabolic energy 12.03 IU; nitrogenous substances 19.5 %; ash 4—6 %; fat 6—8 %; crude fibre max. 4.50 %; nonphytate phosphorus min. 0.40 %; Ca min. 0.85 %; Na min. 0.14 %; retinol 12 500 IU.kg^{-1}; cholecalciferol 3000 IU.kg^{-1}; alfa-tocoferol 40 mg.kg^{-1}. Antioxidants: propylgallat 100 mg.kg^{-1}. Coccidiostats: salinomycinat sodium 70 mg.kg^{-1}.

Composition of BR3 final diet

Maize 37 %; wheat 36.80 %; soybean meal 20 %; limestone 1.12 %; monocalcium phosphate 1 %; salt 0.20 %; lysine 0.98 %; methionine 0.40 %; premix 0.50 %. Chemical composition: metabolic energy 12.37 IU; nitrogenous substances 19 %; ash 4—6 %; fat 6—10 %; crude fibre max. 4.00 %; nonphytate phosphorus min. 0.40 %; Ca min 0.85 %; Na min 0.14 %; retinol 10 000 IU.kg^{-1}; chol ecalciferol 2000 IU.kg^{-1}; alfa-tocoferol 30 mg.kg^{-1}. Antioxidants: propylgallat 100 mg.kg^{-1}.

The control group (GC) was fed with basal diet without any supplement. The experimental group G1 was supplemented in feed with 0.7 % Humac Natur Mycosorb (HNMy). Group G2 was supplemented in feed with 0.3 % Humac Natur Mycosorb (HNMy). The Humac Natur Mycosorb was obtained from HUMAC Ltd. Košice, Slovakia.

Composition of Humac Natur Mycosorb

Powder (particle size up to 100 μm), humic substances 60 %, fulvic acid 5 %, Ca 42.278 $g.kg^{-1}$, Mg 5.100 $g.kg^{-1}$, Fe 19.046 $g.kg^{-1}$, Cu 15 $mg.kg^{-1}$, Zn 37 $mg.kg^{-1}$, Mn 442 $mg.kg^{-1}$, Co 1.24 $mg.kg^{-1}$, Se 1.67 $mg.kg^{-1}$, V 42.1 $mg.kg^{-1}$, Mo 2.7 $mg.kg^{-1}$.

During fattening chickens had access to water and feed *ad libitum*. They were reared on deep litter and microclimatic conditions complied with the requirements for fattening of broilers. The temperature was gradually decreased from 33 °C on day 1 to 21 °C on day 42 and kept constant afterword. The relative humidity was maintained between 50—70 %. After fattening, the animals were stunned and killed by cervical dislocation. Subsequently, breast and thigh muscle samples were taken for further laboratory examination. The muscle samples were immediately frozen and stored at −20 °C until analysed. The analysis consisted of digestion (5 ml HNO_3 and 1 ml HCl per 1g of sample) in a Milestone mineralization system (MLS 1200 Mega) with microwave decomposition technology. Analysis of samples for the presence of calcium, magnesium, copper and zinc was performed on an AAS (Unicam Solar 939, UK) by the flame method (Table 1). The methodology presented in the List of Official Methods and Laboratory Diagnostics of Food and Feed (Bulletin of the Ministry of Agriculture SR, 2004) was used for the determination.

Statistical analysis

The differences between means were determined, according to the unpaired t-test using GraphPad Prism 6 software. Correlations between pairs of elements in each tissue were determined by Pearson correlation analyses. Some of these correlations were highly influenced by the samples that had undetectable mineral concentrations and only samples with detectable mineral levels were included in the analysis. Only significant correlations with an r value > 0.3 are reported.

RESULTS AND DISCUSSION

The values presented in the Tables 2 and 3 are the average values calcium (Ca), magnesium (Mg), copper (Cu) and zinc (Zn) from 6 samples of breast and thigh muscle from each experimental group. The addition of humic substances in feed ensures good animal health and has a positive effect on production parameters and does not require withdrawal periods.

In the breast muscle of broilers from the group G1 we found a statistically significantly higher ($P < 0.05$) Ca content (0.51 $g.kg^{-1}$) compared to the control group (0.43 $g.kg^{-1}$). On the other hand, the addition of 0.3 % HNMy (G2) to feed significantly reduced ($P < 0.05$) Ca content in the thigh muscle (0.32 $g.kg^{-1}$) compared to the control group (0.48 $g.kg^{-1}$), but Ca was slightly increased in the breast muscle (0.47 $g.kg^{-1}$). Probably, the low concentration of Ca in the thigh muscle occurred due to chelating effects of humic substances that are influenced by their large number of carboxylic acid side chains. Mariam et al. [14] found an increased Ca content in poultry meat (1.72 $g.kg^{-1}$) compared to the values found by us. Similarly, Straková et al. [23] when comparing the nutritional content of broiler and pheasant muscle in both sexes, found that higher levels of Ca and Mg in breast muscle (males 2.15; 1.54 $g.kg^{-1}$ and females 2.03; 1.47 $g.kg^{-1}$) and in thigh muscle (males 1.80; 1.12 $g.kg^{-1}$ and females 1.67; 1.13 $g.kg^{-1}$). After feeding humate, increased levels of some essential minerals (such as Ca, Mg, Al and Fe) in serum, liver and muscles were recorded by Stepchenko et al. [22]. The relatively low Ca levels in chicken muscle (13.83 ppm) were

Table 1. Working conditions of atomic absorption spectrometer for analysis of minerals

Element	Fuel	Support	Flame stoichiometer	Wavelength [nm]	Spectral band pass [nm]
Calcium	Acetylene	Air	Oxidizing	422.7	0.5
Magnesium	Acetylene	Air	Oxidizing	285.2	0.5
Copper	Acetylene	Air	Oxidizing	324.8	0.5
Zinc	Acetylene	Air	Oxidizing	213.9	1.0

Table 2. The content of calcium and magnesium in breast and thigh muscle of broilers

Group	Muscle	Ca [g.kg^{-1}]	Mg [g.kg^{-1}]
GC	Breast	0.43 ± 0.07	0.69 ± 0.08
	Thigh	0.48 ± 0.06	0.67 ± 0.12
G1	Breast	0.51 ± 0.09*	0.80 ± 0.06*
	Thigh	0.51 ± 0.12	0.59 ± 0.04
G2	Breast	0.47 ± 0.06	0.73 ± 0.07
	Thigh	0.32 ± 0.06*	0.67 ± 0.12

The data are means of 6 samples of breast and thigh muscles from each group; GC—diet without the addition of humic substances; G1—diet with the addition of 0.7 % Humac Natur Mycosorb; G2—diet with the addition of 0.3 % Humac Natur Mycosorb; *—$P \leq 0.05$

Table 3. The content of copper and zinc in breast and thigh muscle of broilers

Group	Muscle	Cu [mg.kg^{-1}]	Zn [mg.kg^{-1}]
GC	Breast	11.72 ± 1.12	26.62 ± 1.59
	Thigh	11.62 ± 1.18	28.35 ± 4.05
G1	Breast	8.83 ± 1.08**	25.18 ± 2.59
	Thigh	8.50 ± 0.77**	25.75 ± 3.05
G2	Breast	9.35 ± 0.62**	22.45 ± 2.82*
	Thigh	10.07 ± 0.69	24.38 ± 2.85*

The data presented are means of 6 samples of breast and thigh muscles from each group; GC—diet without the addition of humic substances; G1—diet with the addition of 0.7 % Humac Natur Mycosorb; G2—diet with the addition of 0.3 % Humac Natur Mycosorb; *—$P \leq 0.05$; **—$P \leq 0.01$

reported by Ebeledike et al. [4]. A statistically significant increase in Mg content ($P < 0.05$) was analyzed in breast muscle (0.80 g.kg^{-1}) in broilers from the group G1 compared to Mg content in the muscles (0.69 g.kg^{-1}) of broilers from the control group (Table 2).

Differentiated effects have been shown by humic acids to trace elements, especially copper and zinc [8]. Humates can act as a potent metal chelator, it is possible that HA chelates the extracellular ions of some elements and transfers them to the cells, which can be explained as the reason for the increase in concentration.

Our study reports that concentrations of copper and zinc in muscles after the addition of humates were lower compared to values in muscles in control chickens (Table 3). Regarding the essential elements, the average concentrations of the monitored elements in the muscles of chickens in this study did not exceed the maximum permissible limits.

In the control group of broilers, higher levels of Cu were found in the breast and thigh muscles (11.72; 11.62 mg.kg^{-1}) than in the muscles of broilers from the groups G1 (8.83; 8.50 mg.kg^{-1}) and G2 (9.35; 10.07 mg.kg^{-1}). A significant reduction in Cu in the breast and thigh muscles was found in the group G1 ($P \leq 0.01$) compared to the control group. A similar significant reduction ($P \leq 0.01$) was observed only in breast muscle in the group G2. Copper is an element that presents itself as essential but also potentially toxic. The highest concentrations of copper are found in the liver, lower content in the kidneys and the lowest in muscle. Several authors report lower or nearly equal levels

Table 4. Correlation coefficients between minerals (Ca, Mg, Cu, Zn) in the breast muscle (BM) and the thigh muscle (TM) in the control group

Control	Ca BM	Mg BM	Cu BM	Zn BM	Ca TM	Mg TM	Cu TM	Zn TM
Ca BM	1.0	−0.1875	**0.8333**	**0.8159**	0.4450	−0.4808	0.2157	0.3002
Mg BM	–	1.0	−0.1481	−0.4029	0.0745	0.5084	0.2878	**0.7248**
Cu BM	–	–	1.0	0.3919	0.0915	**−0.6771**	−0.3028	0.2873
Zn BM	–	–	–	1.0	0.6061	−0.2322	0.5566	0.0162
Ca TM	–	–	–	–	1.0	−0.1211	0.6754	−0.0255
Mg TM	–	–	–	–	–	1.0	0.5623	0.4637
Cu TM	–	–	–	–	–	–	1.0	0.3339
Zn TM	–	–	–	–	–	–	–	1.0

of the Cu in chicken muscle in Brazil 0.3—3.5 mg.kg^{-1} [6]; in Turkey 0.5 to 12.3 mg.kg^{-1}1 [24] ; 0.27—0.82 mg.kg^{-1} in China [7]. S k a l i c k a et al. [18] recorded in the experiment lower levels of Cu (6.18 0—7.88 mg.kg^{-1}) in breast and thigh muscle (6.13—6.98 mg.kg^{-1}), where the feed mixture was added with the addition of 0.7 % Humac Natur Monogastric with the addition of formates. After iron and zinc, copper is the third most abundant trace element in the body. In Pakistan, M a r i a m et al.[14] monitored copper and zinc concentrations in some animals and found that all sample values in the study were below than the permissible limits. Poultry muscle recorded the lowest concentrations of copper and zinc (12.86 mg.kg^{-1}, 28.52 mg.kg^{-1}, resp.).

In our study we found higher levels of Zn in the breast and thigh muscles (26.62; 28.35 mg.kg^{-1}) than in the muscles of broilers from the groups G1 and G2. The significantly lower content of Zn ($P \leq 0.05$) was found in the breast and thigh muscle of broilers from the group G2 (22.45; 24.38 mg.kg^{-1}) compared to the control group. A decrease in Zn levels in the breast and thigh muscles was also observed in the group G1. K h a n et al. [12] found much higher values in the thigh muscle (107.4 ± 7.60; 106.6 ± 7.37 and 106.78 ± 7.48 mg.kg^{-1}) and breast muscle (107.82 ± 7.66; 107, 4 ± 7.49 and 107.95 ± 7.73 mg.kg^{-1}) taken from three different districts. H u et al. [7] found Zn values of 3.27—17.90 mg.kg^{-1} in the poultry muscle from the food markets region in southern China.

Correlation analysis revealed some relationships between the content of elements in breast and thigh muscles (Table 4).

In the control group, correlations were observed in the breast muscle between Ca and Zn (r = 0.8159) and between Ca and Cu (r = 0.8333). Similarly, positive correlation was observed between Zn in the thigh muscle and Mg in the breast muscle (r = 0.7248). A negative correlation was found for Mg in the thigh muscle and Cu in the breast muscle (r = −0.6771).

The effect of different addition levels of Humac substances on the correlation coefficients of mineral elements in the breast and thigh muscles are shown in Tables 5 and 6.

The calcium content in the breast muscle confirmed a strong positive correlation between the control group and the group G2 (r = 0.7195). The strong positive correlation (r = 0.9093) for Ca was found in the thigh muscle from the control group and in the breast muscle from the group G2. On the other hand, in the group G2, the medium negative correlation was shown (r = −0.6724) for Mg between thigh muscle and breast muscle. Similarly, significantly negative correlation (r = −0.7656) was found between in the thigh muscles, between Mg from the group G2 and Ca from the control group. A high positive correlation (r = 0.7643) were observed in the thigh muscles between Mg content from group G1 and Ca content from the control group.

Other studies examined the relationship between mineral concentrations, and the relationship of mineral concentration physicochemical characteristics in muscles of Japanese Black steers. Magnesium (Mg), potassium (K) and zinc (Zn) concentrations had negative correlations with fat content, but sodium (Na), manganese (Mn), copper (Cu) and molybdenum (Mo) concentrations were not correlated with fat content. The results of the present experiment sug-

Table 5. Correlation coefficients between Ca and Mg in experimental groups in the breast muscle (BM) and the thigh muscle (TM)

		GC			G1				G2			
		Ca TM	Mg BM	Mg TM	Ca BM	Ca TM	Mg BM	Mg TM	Ca BM	Ca TM	Mg BM	Mg TM
GC	Ca BM	0.4450	−0.1875	−0.4808	−0.2282	0.0878	0.5815	0.1301	0.7195	0.6928	−0.0468	−0.3848
	Ca TM	1.0	0.0745	0.1211	−0.4361	−0.5810	0.5292	0.7643	0.9093	−0.0296	0.5504	−0.7656
	Mg BM	–	1.0	0.5084	−0.4079	−0.2611	−0.1884	−0.2331	0.3581	0.1949	0.6002	−0.4305
	Mg TM	–	–	1.0	−0.6571	0.3087	−0.2331	−0.1622	0.0193	−0.3916	0.7616	−0.4236
G1	Ca BM	–	–	–	1.0	−0.2879	−0.5541	0.2185	0.1579	−0.1822	−0.1406	−0.2687
	Ca TM	–	–	–	–	1.0	0.1663	0.0030	−0.5112	−0.0556	−0.4483	0.4957
	Mg BM	–	–	–	–	–	1.0	0.5775	−0.4199	−0.1343	0.3848	−0.2887
	Mg TM	–	–	–	–	–	–	1.0	−0.5853	0.0318	−0.0911	−0.4236
G2	Ca BM	–	–	–	–	–	–	–	1.0	0.1869	0.6189	−0.4726
	Ca TM	–	–	–	–	–	–	–	–	1.0	−0.2383	−0.2407
	Mg BM	–	–	–	–	–	–	–	–	–	1.0	−0.6724

GC—control group; G1—diet with the addition of 0.7 % Humac Natur Mycosorb;
G2—diet with the addition of 0.3 % Humac Natur Mycosorb

Table 6. The correlation coefficients between Cu and Zn in experimental groups in the breast muscle (BM) and the thigh muscle (TM)

		GC			G1				G2			
		Cu TM	Zn BM	Zn TM	Cu BM	Cu TM	Zn BM	Zn TM	Cu BM	Cu TM	Zn BM	Zn TM
GC	Cu BM	−0.3028	0.3919	0.2873	−0.3693	−0.6987	0.7110	0.2207	0.4947	0.5946	−0.7570	−0.1164
	Cu TM	1.0	0.5566	0.3339	−0.1777	−0.0438	−0.7415	0.7003	−0.1334	−0.6652	0.5717	−0.1182
	Zn BM	–	1.0	0.0162	−0.3813	−0.1836	−0.1108	0.9786	0.0519	0.1456	0.0300	−0.3661
	Zn TM	–	–	1.0	0.1577	−0.8666	−0.2526	0.0235	−0.0108	−0.5038	0.0691	−0.1198
G1	Cu BM	–	–	–	1.0	−0.0719	−0.4372	−0.3850	−0.5413	−0.1427	0.3780	0.0054
	Cu TM	–	–	–	–	1.0	0.1395	−0.2734	−0.2188	0.0149	0.3357	0.0972
	Zn BM	–	–	–	–	–	1.0	−0.0907	0.6415	0.8109	−0.9221	0.2245
	Zn TM	–	–	–	–	–	–	1.0	−0.0207	−0.0302	0.1907	−0.3661
G2	Cu BM	–	–	–	–	–	–	–	1.0	0.4836	−0.8403	0.7301
	Cu TM	–	–	–	–	–	–	–	–	1.0	−0.7852	0.2258
	Zn BM	–	–	–	–	–	–	–	–	–	1.0	−0.4701

GC—control group; G1—diet with the addition of 0.7 % Humac Natur Mycosorb;
G2—diet with the addition of 0.3 % Humac Natur Mycosorb

Table 7. Correlation coefficients between minerals (Ca, Mg, Cu and Zn) in experimental group G1 in the breast muscle (BM) and the thigh muscle (TM)

	Ca BM	Mg BM	Cu BM	Zn BM	Ca TM	Mg TM	Cu TM	Zn TM
Ca BM	1.000	–0.5541	–0.4731	0.5100	–0.2879	0.2185	0.7377	–0.1839
Mg BM	–	1.000	–0.0731	–0.1465	0.1663	0.5775	–0.4301	0.6523
Cu BM	–	–	1.000	–0.4372	0.7905	–0.5029	–0.0719	–0.385
Zn BM	–	–	–	1.000	–0.0443	0.1395	–0.1530	-–0.0907
Ca TM	–	–	–	–	1.000	0.0030	–0.0420	–0.4738
Mg TM	–	–	–	–	–	1.000	0.2620	0.3385
Cu TM	–	–	–	–	–	–	1.000	–0.2734
Zn TM	–	–	–	–	–	–	–	1.000

G1—diet with the addition of 0.7 % Humac Natur Mycosorb

Table 8. Correlation coefficients between minerals (Ca, Mg, Cu and Zn) in experimental group G2 in the breast muscle (BM) and the thigh muscle (TM)

	Ca BM	Mg BM	Cu BM	Zn BM	Ca TM	Mg TM	Cu TM	Zn TM
Ca BM	1.000	0.6189	–0.8349	0.6496	0.1869	–0.4726	–0.2770	–0.9277
Mg BM	–	1.000	–0.3096	0.2136	–0.2383	–0.6724	0.0977	–0.6988
Cu BM	–	–	1.000	–0.8403	–0.4381	0.1597	0.4836	0.7301
Zn BM	–	–	–	1.000	0.8228	–0.3821	–0.7852	–0.4701
Ca TM	–	–	–	–	1.000	–0.2407	–0.8698	0.0345
Mg TM	–	–	–	–	–	1.000	0.0636	0.2970
Cu TM	–	–	–	–	–	–	1.000	0.2258
Zn TM	–	–	–	–	–	–	–	1.000

G2— diet with the addition of 0.3 % Humac Natur Mycosorb

gest that mineral concentrations reflect some traits such as fat content but also the composition of myofiber type and the intracellular fluid volume in the muscle [11].

As regards the trace metals, a strong negative correlation was confirmed (r = –0.9221) for Zn content in breast muscle between the groups G2 and G1. A strong positive correlation was observed also between Zn in the breast muscle from the group G1 and Cu in the thigh muscle from the group G2 (r = 0.8109). Similarly, positive correlation was demonstrated between Zn content in thigh muscle from the groups G1 and Zn content in breast muscle from the control group (r = 0.9786). In the group G2 was found positive correlation (r = 0.7301) between Cu content in the breast muscle and Zn content in the thigh muscle and high negative correlation (r = –0.8403) in the breast muscle between Zn content and Cu content in the breast muscle.

S k a l i c k á et al. [20] showed significant correlation between minerals in the liver and thigh muscle. The negative correlation was confirmed between Cd in muscle and Cr in muscle (r = –0.947) and Cu in liver and Cd in muscle (r = –0.885). The results of this study demonstrate antagonism among selected elements.

In addition, significant correlations were observed between copper and most essential elements in breast and thigh muscles of broiler chickens. Tables 7 and 8 summarise the correlation analysis between minerals in breast and thigh muscles in experimental groups G1 and G2.

After addition of 0.7 % HNMy to diet we observed a strong positive correlation between Cu content in the breast muscle and Ca content in the thigh muscle (r = 0.7905). Also between Cu content in the thigh muscle and Ca in the breast muscle (r = 0.7377). S k a l i c k á et al. [21] in the study with addition 0.7 % Humac Natur in the diet for broiler chickens were found negative correlation between the elements: Ca and Cu (r = –0.6582) in the breast muscle.

On the contrary, after addition of 0.3 % HNMy to the diet we observed negative correlations in the thigh muscle between copper and calcium (r = –0.8698). A strong negative correlation was observed between Zn content in the thigh muscle and Ca content in the breast muscle (r = –0.9277). On the other hand, in the study with 0.5 % Humac Natur in the diet there was a positive correlation between Cu in the thigh muscle and Ca in the breast muscle (r = 0.8881) [21].

CONCLUSIONS

Increased attention as an alternative to feeding antibiotics in poultry production has been paid to ecological additives. Organic additives are more acceptable by consumers. According to the results of this experiment, the use of the 0.7 % and 0.3 % Humac Natur Mycosorb as feed supplement contributed to the increase in the Ca and Mg content in the breast and thigh muscles of broilers. Changes in concentrations of elements observed in the muscle of chickens after the addition of humates included in this study were caused by mutual interactions. The mechanism involves formation of chelate bonds with the elements. To sum up, the Humac Natur can be considered a good feed supplement which positively affects the nutritional value of chicken meat.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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