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Effect of humic substances on the mineral composition of chicken meat

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Abstract

The study was conducted to determine the concentrations of mineral substances calcium (Ca), magnesium (Mg), copper (Cu), and zinc (Zn) in the thigh and breast muscle of broiler chicks after application of humic substances. Eighty-one-day-old broiler chicks were randomly divided into 4 groups of 20 animals. Group 1 (G1) was supplemented with 0.7% Humac Natur (HN), G2 with 0.7% Humac Natur Monogastric (HNM) and G3 with 0.5% HNM and control group (CG) received basal diet without any supplements. The samples of muscles were analyzed using flame atomic absorption spectrometry method (AAS). The statistically significantly higher levels of Ca and Zn ($p<0.01$) were found in breast and thigh muscles in G1 in comparison to the CG. An addition of the 0.7% HNM in G2 caused the significantly higher levels of the Ca ($p<0.05$) and Zn ($p<0.01$) in both types of muscles in comparison to the CG. The addition of 0.7% HNM ($p<0.05$) and 0.5% HNM ($p<0.01$) in feed significantly reduced the amount of the Cu in the breast muscle versus the addition of 0.7% HN. Significantly lower Zn contents ($p<0.01$) were found in the breast and thigh muscle of broilers of CG in comparison to the G1 with addition of 0.7% HN. In the group G1 was achieved a strong positive correlation in the thigh muscle between the Mg and the Zn ($r=0.9660$) after application of 0.7% HN. According to the results, significant positive correlation between the Zn and the Mg ($r=0.8187$) in the breast muscle was observed in the group G2. In conclusion, HN and HMN can be considered a good additive with a positive impact on the nutrition value of poultry meat.

Introduction

Chicken meat is relatively inexpensive because chickens can be produced faster and with higher feed efficiency than other commercial meats. Meat is a popular ingredient in our diet, people consume it mainly for its organoleptic qualities (color, taste), but also for nutritional reasons - full of protein, vitamins and minerals. Meat quality can be influenced by handling the feeding of broiler chickens [1] as well as *postmortem* treatment of the carcass. Since the ban on feeding antibiotics fodder, the subject of research has been to add additives made from extracts, vegetable oils derived from various spices, herbs, bio-fermented feeds and humic substances. Humic substances are naturally occurring organic compounds resulting from the decomposition of plant and animal residues. They show antibacterial, antiviral, antithyroid and anti-inflammatory effects in animals, improve immunity and reduce mortality and increase poultry growth [2]. Arif *et al.* [3] describes useful applications and aspects of humic substances, including their action and methods for their production and protection of animal and poultry health.

Humic substances have shown strong affinity for binding various substances, such as heavy metals [4], minerals [5] and aflatoxins [6,7]. Feeding humic substances increased levels of some essential minerals (such as Ca, Al and Fe) in serum, liver and poultry muscles [8]. Differentiated effects showed humic acids to trace elements, especially Cu and Zn [2]. Zn, Cu, and Mn are essential trace elements and play multiple biological and physiological roles in the development and health of all animals [9] and they are confirmed mutual interactions among these elements [10]. Several authors have been involved in the appearance of some elements in wild birds and poultry breeding [11-13]. As a potential public health concern, residues of trace metals in chicken meat products have been studied in various countries and regions [14-17]. The aim of this work 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.

Material and method

Animals

Eighty-one-day-old broiler chicks COBB 500 (MACH DRUBEŽ Ltd. Litomyšl, Czech Republic) were randomly divided into 4 groups of 20 animals each. Dietary treatments were as follows: The broilers were fed with 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 fed mixture (AGROCASS plus, Ltd. Čaňa, Slovak Republic) for the duration of the experiment (38 days).

Experimental design and diet

The control group (CG) was fed with basal diet without any supplement. The experimental group G1 was supplemented with 0.7% Humac Natur (HN) which represented 0.7 kg /1000 kg feed. Group G3 was supplemented with 0.7% Humac Natur Monogastric (HNM) which represented enriched in calcium formate. Group G4 was supplemented with 0.5% Humat Natur Monogastric (0.5 g /1000 kg feed). The Humac Natur and Humac Natur Monogastric was obtained from HUMAC Ltd. Košice, Slovak republic. During fattening

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chickens have access to water and feed *ad libitum*. The broiler chicks were reared on deep litter and microclimatic conditions complied with the requirements for fattening of broilers. Broilers were reared under a conventional temperature regiment at 21^{0}C. The relative humidity was maintained between 60-70%. After fattening, the animals were stunned and killed by cervical dislocation. Subsequently, breast and thigh muscle samples were taken for further laboratory examinations. The experiment was carried out in accordance with the European directive on the protection of vertebrate animals used for experimental and other scientific purposes (86/609/EU) and with the consent of the State Veterinary and Food Administration of the Slovak Republic No. 3090/13-221 in the premises in poultry housing of the University of Veterinary Medicine and Pharmacy in Košice (Slovak republic).

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%; Premix0.50%

Chemical composition: Metabolic energy 12.01 MJ; Nitrogenous substance 22%; Ash 6%; Fat 2.5 – 5.0% ; Crude fibre max 4.00%; Non-phytate phosporus min 0.42%; Ca min 0.9%; Na min 0.15%; Retinol 12 500 mj/kg; Cholecalciferol 3 000 mj/kg; Alfa-tocoferol 50mg/kg; Antioxidants: Propylgallat 100 mg/kg; Coccidiostats: Narazin 70mg/kg.

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 MJ; Nitrogenous substance 19.5%; Ash 4–6%; Fat 6 – 8%; Crude fibre max 4.50%; Non-phytate phosphorus min 0.40%; Ca min 0.85%; Na min 0.14%; Retinol 12 500 mj /kg; Cholecalciferol 3 000 mj/kg; Alfa-tocoferol 40 mg/kg; Antioxidants: Propylgallat 100 mg/kg; Coccidiostats: Salinomycinat sodium 70 mg/kg.

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 MJ; Nitrogenous substance 19%; Ash 4–6%; Fat 6–10%; Crude fibre max 4.00%; Non-phytate phosphorus min 0.40%; Ca min 0.85%; Na min 0.14%; Retinol 10 000 mj /kg; Cholecalciferol 2 000 mj/kg; Alfa-tocoferol 30 mg/kg; Antioxidants: Propylgallat 100 mg/kg.

Composition of Humac Natur (HN)

powder (particle size up to 100μm) with Humic substances 60%, Fulvic acid 5%, Ca 42.278 g/kg, Mg 5.100 g/kg, Fe 19.046 g/kg, Cu 15 mg/kg, Zn 37 mg/kg, Mn 442 mg/kg, Co 1.24 mg/kg, Se 1.67 mg/kg, V 42.1 mg/kg, Mo 2.7 mg/kg.

Composition of Humac Natur Monogastric (HNM)

powder (particle size up to 100μm) with Humic substances 60%, Fulvic acid 5%, Formic acid 3.24%, Ca 0.0511 g/kg, Mg 4.855 g/kg, Fe 18.094 g/kg, Cu 14.25 mg/kg, Zn 35.15 mg/kg, Mn 135 mg/kg, Co1.18 mg/kg, Se1.59 mg/kg, V 40 mg/kg, Mo 2.57 mg/kg.

Sample preparation

The samples of the muscles were immediately frozen and stored at -20 ^{0} C until analysis. The analysis consisted of a digestion in the microwave oven (MLS-1200 Mega, Milestone) by using 5mL HNO_3 and 1 mL HCl pre 1g of sample. The digested samples were analysed for the presence of Ca, Mg, Cu, and Zn using a flame atomic absorption spectrometry (Unicam Solar, 939). The methodology used for the determination of mineral elements is presented in the List of Official Methods and Laboratory Diagnostic of Food and Feed in Bulletin of the Ministry of Agriculture of the Slovak Republic, 2004.

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.

Result and discussion

In our study, we have assumed that potential synergistic effects between minerals and humic acid could result in beneficial effect on nutrition condition of muscles. Application of humic substances in feed or drinking water ensures good animal health and positively affects production parameters. The application of humic substances for animals in feed does not require withdrawal periods. The average concentrations of Ca, Mg, Cu and Zn in broiler muscles are presented in Table 1.

Calcium

The statistically significantly higher ($p<0.01$) Ca content was found in the breast and thigh muscle of broilers fed with additions 0.7% HN (0.93; 0.91 g.kg^{-1}) in comparison to the control group. Similarly, higher Ca ($p<0.05$) content was in muscles also in broilers fed with additions 0.7% HNM (0.89; 0.78 g.kg^{-1}) in comparison to the control group (0.42; 0.31 g.kg^{-1}). A lower significant ($p<0.01$) Ca content was found in the breast and thigh muscle of the broilers fed with 0.5% HNM (0.42; 0.31 g.kg^{-1}) in comparison to the experimental broiler groups fed with 0.7% HN and 0.7% HNM. The lower level of Ca the breast (0.35 g.kg^{-1}) and thigh (0.49 g.kg^{-1}) muscle of broilers after addition of 1% in the feed Humac Nature analyzed Skalicka *et al*. The relatively low Ca levels in chicken muscle (13.83 ppm) was presented at work from Ebeledike *et al.* [18]. Higher Ca content in poultry meat (1.72 g.kg-1) was found by

Table 1. The content of mineral elements in breast and thigh muscle of broiler chickens

Group	Muscle	Ca g.kg^{-1}	Mg g.kg^{-1}	Cu mg.kg^{-1}	Zn mg.kg^{-1}
Control	breast	0.42±0.06	0.47±0.03	6.67±1.58	16.85±1.71
	thigh	0.31±0.10	0.41±0.04	7.03±1.25	14.42±1.52
0.7% HN	breast	0.93±0.31**	0.53±0.03**	7.88±0.60	23.38± 3,34**
	thigh	0.91±0.30**	0.43±0.03**	6.98±0.06	21.70±3.92**
0.7% HNM	breast	0.89±0.33**	0.53±0.04**	6.18±1.61*	23.30±2.55**
	thigh	0.78±0.36*	0.40±0.01	6.13±0.69	21.72±2.27**
0.5% HNM	breast	0.33±0.08**	0.45±0.04	5.80±0.96	18.32±2.28
	thigh	0.34s±0.07**	0.35±0.04	7.03±1.85	20.55±3.37**

The data represents the mean of 6 samples of breast and thigh muscles from each group; Control group – diet without the addition of humic substances; 0.7% HN – diet with the addition of Humac Natur; 0.7% HNM – diet with the addition of Humac Natur Monogastric; 0.5% HNM – diet with the addition of Humac Natur Monogastric; *P≤0.05 ; **P≤0.01.

Mariam *et al.* [19]. Similarly, Straková *et al.* [20] when compared the nutritional content of muscle of broilers and pheasants in both sexes found that much higher levels of Ca and Mg in the breast muscle (males 2.15; 1.54 $g.kg^{-1}$ and females 2.03; 1.47 $g.kg^{-1}$) also in thigh muscle (male 1.80; 1.12 $g.kg^{-1}$ and female 1.67; 1.13 $g.kg^{-1}$).

Magnesium

A statistically significant increase ($p<0.01$) of Mg was analyzed in the breast (0.53 $g.kg^{-1}$) and thigh (0.43 $g.kg^{-1}$) muscle in the broiler chickens fed with addition 0.7% HN in comparison to control group. Similarly, statistically significant increase ($p<0.01$) was found in the breast muscle after addition of 0.7% HNM (0.53 $g.kg^{-1}$) versus the content of Mg in the breast muscle (0.47 $g.kg^{-1}$) and thigh muscle (0.41 $g.kg^{-1}$) of the broiler in the control group and in comparison to Mg content of the breast muscle (0.45 $g.kg^{-1}$) and thigh (0.35 $g.kg^{-1}$) broilers fed with addition of 0.5% HNM. The Mg content in the muscles of the broiler chickens remained at a similar level, regardless of the application and the amount of the supplement. Introduction of additives as humic substances to poultry diet may exert an effect on the level of element retention or interaction in tissues and their status in the organism. The favourable mechanisms of humic substances include alteration of the gastrointestinal functions, induction and inhibition of metabolic enzymes and transport proteins, beneficial modification of the intestinal microbiota, increased digestibility and nutrient absorption [21].

Copper

In the control group of broilers, almost equal average Cu levels were found in the breast and thigh muscles (6.67; 7.03 $mg.kg^{-1}$), as in broiler muscles from the groups fed with addition of 0.7% HN (7.88; 6.98 $mg.kg^{-1}$), 0.7% HNM (6.18; 6.13 $mg.kg^{-1}$) and 0.5% HNM (5.80; 7.03 $mg.kg^{-1}$). The addition of 0.7% HNM ($p<0.05$) and 0.5% HNM ($p<0.01$) in feed significantly reduced the amount of Cu in the breast muscle versus the addition of 0.7% HN. Much higher amounts of the Cu were found in muscles (13.16; 16.50 $mg.kg^{-1}$) fed with addition of 1% Humac Natur. Several literatures reports lower Cu levels in chicken muscle in Brazil 0.3 - 3.5 $mg.kg^{-1}$ [22]; 1.00-1.13 $mg.kg^{-1}$ in Nigeria [23]; in Turkey 0.5-12.3 $mg.kg^{-1}$ [24]; 0.27 - 0.82 $mg.kg^{-1}$ in China [11]. The discrepant results concerning the effect of additives on lower levels or retention of trace element in muscles in production animals are probably related to the different content of phytochemicals.

Zinc

In our study we found (16.85; 14.42 $mg.kg^{-1}$) significantly lower Zn contents ($p<0.01$) in the breast and thigh muscle of broilers fed with no feed additive in comparison to the addition of 0.7% HN (23.38; 21.70 $mg.kg^{-1}$) in experimental group and in groups with addition of 0.7% HNM (23.30; 21.72 $mg.kg^{-1}$) and 0.5% HNM in only the thigh muscle (20.55 $mg.kg^{-1}$). The result of our work was different from that done by Akan *et al.* [25] who found the Zn concentration (1.1 ppm) in chickens. Mariam *et al.* [19] found similar Zn levels in the muscles (28.52 ppm) of poultry. In Pakistan, Khan *et al.* [26] found much higher values in chicken thighs (107.4 ± 7.60; 106.6 ± 7.37 and 106.78 ± 7.48 ppm) and breasts (107.82 ± 7.66; 107.4 ± 7.49 and 107.95 ± 7.73) taken from three different districts. Lower levels of Zn in the breast and thigh muscles (15.14; 19.50 $mg.kg^{-1}$) were recorded after administration of 1% Humac Natur in broiler feed [27]. Hu *et al.* [11] found Zn values of 3.27 - 17.90 in the poultry muscle from the food markets region in southern China.

Correlation analysis revealed some relationships between the contents of elements in breast and thigh muscle (Table 2).

In the control group, correlation relations were manifested between Ca and Zn (r=0.6399) in the thigh muscle and also in the case of Cu in the thigh muscle to Zn in the breast muscle (r=0.8563). Significantly negative correlation was found in the Mg between the muscles (r=-0.8865). Similarly, negative correlation was observed in the breast muscle between Ca and Cu (r=-0.7447).

The effect of different addition levels of Humat Natur substances on the correlation coefficients of mineral elements in the breast and thigh muscles are shown in Tables 3-5.

The results show that the addition of 0.7% of HN in the feed caused a strong positive correlation in the thigh muscle between the Mg and

Table 2. The correlation coefficients between mineral elements (Ca, Mg, Cu, Zn) in the breast muscle (BM) and thigh muscles (TM) in control group

Control	Ca BM	Mg BM	Cu BM	Zn BM	Ca TM	Mg TM	Cu TM	Zn TM
Ca BM	1.0	0,3501	**-0,7447**	0.0784	-0.2205	-0.4221	0.2573	-0.0576
Mg BM		1.0	-0.0606	0.2190	-0.1377	**-0.8865**	0.5388	**-0.6572**
Cu BM			1.0	0.5191	-0.0089	0.4033	0.2592	-0.3540
Zn BM				1.0	-0.3049	0.2360	**0.8563**	-0.3515
Ca TM					1.0	0.0661	-0.5753	**0.6399**
Mg TM						1.0	-0.1781	0.4731
Cu TM							1.0	-0.5793
Zn TM								1.0

Table 3. The correlation coefficients between mineral elements (Ca, Mg, Cu, Zn) in the breast and thigh muscles with additions 0.7% Humat Natur (HN)

0.7% HN	Ca BM	Mg BM	Cu BM	Zn BM	Ca TM	Mg TM	Cu TM	Zn TM
Ca BM	1.0	0.1763	**-0.6582**	-0.5411	**0.6703**	0.5600	0.3772	0.5606
Mg BM		1.0	-0.0324	-0.5175	0.3138	0.6950	0.4792	0.6070
Cu BM			1.0	0.2644	-0.4259	-0.4508	-0.3135	-0.5875
Zn BM				1.0	-0.1017	-0.1680	**-0.6849**	-0.1501
Ca TM					1.0	**0.7092**	0.4940	0.5815
Mg TM						1.0	0.2054	**0.9660**
Cu TM							1.0	0.0951
Zn TM								1.0

Table 4. The correlation coefficients between mineral elements (Ca, Mg, Cu, Zn) in the breast muscle (BM) and thigh muscles (TM) with additions 0.7% Humat Natur Monogastric (HNM)

0.7%HNM	Ca BM	Mg BM	Cu BM	Zn BM	Ca TM	Mg TM	Cu TM	Zn TM
Ca BM	1.0	-0.3276	0.3125	-0.0516	-0.2834	0.2316	-0.0516	-0.4333
Mg BM		1.0	-0.5292	**0.8187**	-0.5001	0.2857	-0.5936	**-0.6958**
Cu BM			1.0	-0.2288	-0.2157	-0.3787	**0.7076**	0.3775
Zn BM				1.0	**-0.6972**	0.1140	-0.6141	-0.6603
Ca TM					1.0	-0.2702	0.0067	0.5930
Mg TM						1.0	0.1566	0.2690
Cu TM							1.0	**0.6387**
Zn TM								1.0

Table 5. The correlation coefficients between mineral elements (Ca, Mg, Cu, Zn) in the breast muscle (BM) and thigh muscles (TM) with additions 0.5% Humat Natur Monogastric (HNM)

0.5%HNM	Ca BM	Mg BM	Cu BM	Zn BM	Ca TM	Mg TM	Cu TM	Zn TM
Ca BM	1.0	0.3290	0.5325	**0.6446**	**-0.6191**	-0.1518	**0.8881**	0.3534
Mg BM		1.0	-0.0465	0.5255	0.2683	-0.5103	0.2146	-0.0249
Cu BM			1.0	0.1334	-0.3730	0.1709	**0.8450**	0.4763
Zn BM				1.0	-0.3061	0.0982	0.5263	**0.7077**
Ca TM					1.0	0.1804	**-0.6180**	-0.2989
Mg TM						1.0	-0.0395	**0.6140**
Cu TM							1.0	0.5207
Zn TM								1.0

the Zn (r=0.9660) and Mg and Ca (r=0.7092). A positive correlation was confirmed in Ca content between the muscles (r=0.6703.) The breast muscle was demonstrated with negative correlation between the elements: Ca to Cu (r=-0.6582). The negative correlation was found between Zn content in the breast muscle and Cu content in the thigh muscle (r=-0.6849).

According to the results, broilers fed 0.7% of HNM showed a significant positive correlation in the breast muscle between Zn and Mg (r=0.8187) as well as in the thigh muscle between Cu and Zn (r=0.6387). The positive correlation (r=0.7076) was confirmed in Cu in the breast versus the thigh muscle. The negative correlation was found between the breast and thigh muscle in the elements: Mg in the breast muscle to Zn in the thigh muscle (r=-0.6958) and Zn in the breast muscle to Ca in the thigh muscle (r=-0.6972).

The positive correlation was also found in the broiler muscles of chicken fed 0.5% of HNM. It was recorded in the breast muscle between Ca and Zn (r=0.6446), in the thigh muscle between Mg and Zn (r=0.7077). Similarly, the positive correlation was observed in Cu from the thigh muscle to Ca from the breast muscle (r=0.8881) and to Cu in the breast muscle (r=0.8450). Negative correlation was occurred in Ca between the thigh muscle versus the breast muscle (r=-0.6191) and Cu in the thigh muscle (r=-0.618). Skalická *et al.* [28] found significant correlation relationships 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.

Conclusion

According to the results of this experiment, the use of the 0.7% Humat Natur (HN) and 0.7% Humat Natur Monogastric (HNM) as supplement in the feed had significantly effect and contributed to the increase in the Ca and Zn content in the breast and thigh muscles of broilers chicks. There is a need for further investigations to elucidate the mechanisms. This is related to the ability of humic substances and its formation of chelate bonds with elements. To sum up, the Humac Natur can be considered as a good feed supplement, which can have positive effects on the nutritional value of chicken meat.

Implications

Increased attention as an alternative to feed antibiotics in poultry production is complemented by ecological additives. These organic additives are more acceptable among consumers. The addition of HA in broiler diets improved the palatability and quality of poultry meat.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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