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CHEMICAL COMPOSITION OF CHICKEN MEAT AFTER APPLICATION OF HUMIC ACID AND PROBIOTIC *LACTOBACILLUS FERMENTUM*

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ABSTRACT
The aim of the present study was analysed and evaluated chemical parameters of chicken breast and thigh muscles after addition of humic acids and probiotic into diet for broiler chicken. A total of 200 pcs Ross 308 broiler chickens were divided into 4 groups (n=50). The control group of chickens was fed with complete feed mixtures without any additives. Chickens in experiment groups were fed a diet containing: P1 (1% of humic acid), P2 (1% of humic acid and probiotic supplement *Lactobacillus fermentum*) and P3 were fed with complete feed mixture containing combination of starter feed mixture (1. – 21. day) with coccidiostaticum Diclazuril and growth feed mixture (21. – 35. day) containing Salinomycinum sodium. Besides, the groups were kept under the same conditions. Fattening period lasted for 42 days. Chicken meat was analyzed for content of water, crude protein, fat and cholesterol. Based on the results, we can state that the application of humic acids or the combination of Humac Natur with probiotic did not affect the chemical composition of the breast muscle. In the breast muscle, the protein content in the experimental group P3 with the coccidiostat (22.98 $g.100\ g^{-1}$) was reduced ($p \leq 0.05$) compared to control group (23.42 $g.100\ g^{-1}$). In the case of thigh muscle was significantly higher content of fat and cholesterol ($p \leq 0.05$) in chickens feeding with addition of Humac Natur (fat – 9.08 $g.100g^{-1}$; cholesterol – 0.86 $mg.100g^{-1}$) and similar results were recorded in experimental group with combination of Humac Natur and probiotic (fat – 9.15 $g.100g^{-1}$; cholesterol – 0.86 $mg.100g^{-1}$) compared to control group (fat – 7.15 $g.100g^{-1}$; cholesterol – 0.70 $mg.100g^{-1}$). From a general point of view, we can recommend the application of Humac Natur, respectively combination Humac Natur with probiotics in feeding of broiler chickens Ross 308.

Keywords: chemical composition; chicken meat; humic acids; dietery supplemation

INTRODUCTION
Meat quality has always been important to the consumer, and it is an especially critical issue for the meat industry in the 21st century **(Joo et al., 2013)**. High product quality and food safety are key targets for the food industry, since they relate to customer satisfaction and ultimately to repeat purchase **(O'Sullivan, 2017)**. In addition, the aim of food researchers and producers is to increase the nutritional value of food without decreasing sensory quality or consumers' acceptability **(Miezeliene et al., 2011)**. Poultry meat represents an important component of human diet. Complete feed mixtures for broiler chickens are often enriched with various additives as vegetable oils, probiotic, prebiotic and enzyme preparations **(Lee et al., 2003, 2004; Shalmany and Shivazad, 2006)**. After the application of antibiotics as feed additives in order to enhance growth in production animals has lately been restricted **(Enberg et al., 2000),** researchers have looked for new feed additives that are not harmful to human health.

The importance of these alternative supplement consists mainly in the replacement of antibiotics and coccidiostats which were banned by the European Union since 2006 for poultry husbandry. Several additives have been tested as growth promoters to avoid the excessive use of antibiotics or at least reduce or substitute their inclusion in feeds, while maintaining an efficient animal production to obtain safe edible products **(Islam et al., 2005; Gomez et al., 2012)**. The use of most antibiotic growth promoters has been banned in many countries, because it is risky due to cross resistance amongst pathogens and residues in tissues. Humic acids, one of the potential substances alternatives to antibiotics in the diet of poultry, are formed from decayed plant matter with the aid of living bacteria in the soil **(Nagaraju et al., 2014)**.

The basic problems of poultry feeding consist not only of sufficient supply of the main feed materials **(Kluth and Rodehutscord, 2006; Gous, 2010),** but also of looking for safe and native food sources **(Corzo et al., 2005; Brenes and Roura, 2010)**. The humic substances are very common in nature as they originate from the decomposition of organic matter, and are normally present in the drinking water and soil **(Islam et al., 2005)** and humic acid shows antibacterial, antiviral, antithyroideal and antimicrobial effects in animal husbandry to improve the economy and ecology of animal production by increasing growth rate, decreasing feed expenditure per gain and diminishing the risk of disease **(Eren et al., 2000; Kocabagli et al., 2002; Rath et al., 2006)**. Humic acid is known to be nontoxic and nonteratogenic and has been used as analgesic and antimicrobial agent in veterinary practices **(Yasar et al., 2002)** and humic acid based mixtures have the potential to be an alternative to antibiotic growth promoters in broiler diets **(Ceylan et al., 2003)**.

Humic acids are organic compounds naturally present in water and soil. They form three-dimensional structure molecules, containing aromatic nuclei with oxygen and nitrogen heterocycles. In the side chains, bound to an aromatic nucleus, hydroxyl, carbonyl, carboxyl, amine and sulfhydryl functional groups are present **(MacCarthy 2001; Zralý et al., 2008)**.

Ozturk et al. (2010) found that the humic acids had positive effect on growth, meat quality, carcass characteristics, selected parameters determined in the blood and in the gastrointestinal tract. Humic acids are naturally occurring decomposed organic constituents of soil and lignite that are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains **(MacCarthy, 2001)**. The use of humic acid to replace antibiotics in poultry has gained widespread interest **(Mutus et al., 2006)**. It has been observed that humic acid included in the feed and water of poultry promote growth **(Kocabagli et al., 2002; Mirnawati and Marlida, 2013)**. The humic substances had positive effect on the growth of animals and feed conversion **(Kocabağli et al., 2002; El-Husseiny et al., 2008; Ozturk et al., 2012; Mirnawati and Marlida, 2013)**.

As diet is one of the most important factors affecting meat quality **(Tateo et al., 2013)**, various benefits in regard to meat quality characteristics can be gained by supplementing broiler diets, particularly using probiotics as feed additives **(Karaoglu et al., 2004)**. Among the possible alternatives, probiotics are considered a promising alternative to antibiotics, as well. Probiotic is defined as a live microbial feed supplement that beneficially affects the host animal by improving the intestinal microbial balance **(Alkhalf et al., 2010; Daneshmand et al., 2015)**. Probiotics cover a wide range of living microorganisms with supposed positive effects on gut flora and producing many substances supporting many different effects **(Bernardeau and Vernoux, 2013)**. Probiotics are live, non-pathogenic bacteria that contribute to the health and balance of the intestinal tract **(Giannenas et al., 2012; Bajaj et al., 2015; Uyeno et al., 2015)**. Several studies showed that dietary supplementation of lactic acid bacteria (e.g. *Lactobacillus*) improve the performance and feed conversion **(Taklimi et al., 2012; Bai et al., 2013)**. The enhanced growth with probiotics may be partly attributed to the colonisation of the gastrointestinal tract of the chicks, which improved the digestion of essential nutrients **(Khaksefidi and Rahimi, 2005)**. Various studies have reported a wide variety of health-promoting properties influencing the host intestinal balance **(Shim et al., 2012; Blajman et al., 2015)**, as well as quality of chicken eggs **(Zhang et al., 2012; Angelovičová et al., 2013)** and chicken meat **(Abdel-Latif et al., 2008; Bobko et al., 2015; Haščík et al., 2016; Wang et al., 2017)**.

Scientific hypothesis

This study was designed to investigate the effects of dietary addition of humic acids or combination of humic acids with probiotic preparation based on Lactobacillus fermentum on meat chemical composition of Ross 308 broiler chickens. We expect that addition of these preparations will have a positive affcet on chosen parameters of chemical composition of broiler meat.

MATERIAL AND METHODOLOGY

Animal and diets

The experiment was realized at the Department of Poultry Science and Small Farm Animals in the experimental poultry house on College farm in Kolíňany.

In every experiment a total 200 one-day-old Ross 308 meat hybrid chicken was included. Chickens were randomized into four groups, each containing 50 birds. Chickens in individual groups were stabled on deep budding, with a maximum occupation of the breeding areas 33 kg.m^{-2}. During the fattening period, the light regimen based on 23 h of light and 1 h of dark was used.

The temperature at the beginning of the experiment was 31 – 33 °C and decreased to 20 – 22 °C during the experiment. The temperature was maintained using electronic hen-like devices providing radiant heat.

The fattening lasted 42 days. The feeding program included three phases: starter (1st – 21st days of age), grower (22nd – 35th days of age), and finisher (36th – 42nd days of age). Feed and water were supplied *ad libitum.* Composition of complete feed mixtures is presented in Table 1.

In control group we used complete feed mixture without any additives. Group of chickens P1 was fed a diet containing 1% of preparation Humac Natur. The group marked as P2 was fed a diet containing 1% of preparation Humac Natur and probiotic supplement Propoul in water (0.03 g per pc) and group P3 containing combination of starter feed mixture with coccidiostaticum Diclazuril (each kg contains 5 g of Diclazuril) and growth feed mixture containing Salinomycinum sodium (each kg contains 120 g of Salinomycin).

In the experiment, the probiotic preparation "Propoul" based on *Lactobacillus fermentum* (1.10^{9} CFU per 1 g of bearing medium) was used.

Humac Natur purchased from Humac s.r.o., Kosice is preparation of humic substances on base of oxihumolit contain min. 62% humic acids in dry matter, of this 48% free munic acids in dry matter, minerals and trace elements, carboxymethylcellulose complex with humic substances. Moisture was maximum 11%.

Slaughter and measurements

At 42 days of age, chickens were weighed and slaughtered at the experimental slaughterhouse of Slovak University of Agriculture in Nitra. The chemical analysis of chicken meat (breast muscle without skin, thigh muscle with skin and subcutaneous fat) for analyse of crude protein, fat, wate and cholesterol content, was performed using an Infratec 1265 Meat Analyzer.

Statisic analysis

A statistical analysis was computed using the ANOVA procedures of SAS software with using of Enterprise Guide.

4.2 application (version 9.3, SAS Institute Inc., USA, 2008). Data were reported as mean ± standard deviation. Statistical significance was calculated using t-test. Differences between the groups were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of experiment with Ross 308 broiler chickens after addition of humic acid and probiotic, which was aimed at analysed and evaluated chemical parameters, are presented as follows: the results of crude protein, fat, water and cholesterol content in breast and thigh muscle are given Table 2.

Humic acids stabilize the intestinal flora and thus ensure an improved utilization of nutrients in animal feed **(Islam et al., 2005)**.

The higher average value of crude protein content measured in fresh breast muscle after addition of supplements was in control group C without addition (23.42 g.100 g^{-1}) and the lowest value was measured in experimental group P2 with addition of humic acid and probiotic (22.49 g.100 g^{-1}). We have found statistically significant differences ($p \leq 0.05$) between control group C and experimental groups P2 and P3.

In the case of fresh thigh muscle was measured the lowest value of crude protein content 18.70 g.100 g^{-1} in experimental group P2 with combination of humic acid and probiotic *Lactobacillus fermentum* and the higher value in experimental group P3 containing combination of starter feed mixture with coccidiostaticum Diclazuril and growth feed mixture containing Salinomycinum sodium (19.93 g.100 g^{-1}). We have found statistically significant differences ($p \leq 0.05$) between experimental group P1 and experimental groups P2 and P3.

Ozturk et al. (2010) evaluated the chemical composition of breast and thigh muscles of broiler chickens Ross 308 after addition of humic substances (0.5; 1.0 and 1.5 %) into fed. Content of protein was the higher in control group 22.94% and the lowest in group with addtiotion 1% of humic substances (22.49%). In the thigh muscles, content of protein was 17.32% in cotrol group and 17.44% in group with 1% of humic substances.

Ozturk et al. (2011) reported that addition of 1% humic substance into fed for broiler chickens Ross 308 decreased the protein content of thigh meat in relation to control group and 1.5% humic substance, and the protein content of breast meat compared to control group ($p < 0.10$).

The effect of adding humic acids on the quality of the meat of broiler chickens Cobb 500 was monitored in their work **Reitznerová et al. (2016)**. They found out that content of protein in breast muscle was 23.36% compared to control group (23.52%) and in thigh muscle was 19.76% compared to 20.16% in control group.

Ondruška et al. (2012) evaluated the addition of combination Humac Natur (0.3%) and probiotic *L. fermentum* on rabbit meat quality. Content of total protein was in control group 22.07 g.100 g^{-1} compared to tested group 21.70 g.100 g^{-1}.

In evaluating of fat content in fresh breast muscle was found the lower value 0.84 g.100 g^{-1} in experimental group P1 with addition of humic acid and the higher average value 1.96 g.100 g^{-1} in group with addition of Humac Natur and probiotic (experimental group P2). In thigh muscle was found the higher value of fat content in experimental group P2 (9.15 g.100 g^{-1}) and the lower average value 7.15 g.100 g^{-1} in experimental group P1. We have found statistically significant differences ($p \leq 0.05$) in breast

Table 1 Composition of feed mixtures.

Ingredients (%)	Starter (HYD-01) (1^{st} – 21^{st} day of age)	Grower (HYD-02) (22^{nd} – 35^{th} day of age)	Finisher (HYD-03) (36^{th} – 42^{nd} day of age)
Wheat	35.00	35.00	36.82
Maize	35.00	40.00	37.00
Soybean meal (48% N)	21.30	18.70	20.00
Fish meal (71% N)	3.80	2.00	-
Dried blood	1.25	1.25	-
Ground limestone	1.00	1.05	1.10
Monocalcium phosphate	1.00	0.70	1.00
Fodder salt	0.10	0.15	0.20
Sodium bicarbonate	0.15	0.20	0.25
Lysine	0.05	0.07	0.29
Methionine	0.15	0.22	0.29
Palm kernel oil Bergafat	0.70	0.16	2.50
Premix Euromix BR 0.5%*	0.50	0.50	0.50
Nutrient composition (g.kg^{-1})			
Linoleic acid	13.51	14.19	149.1
Fibre	30.18	29.93	30.54
Crude protein	210.76	190.42	170.58
MEN (MJ.kg^{-1})	12.01	12.03	12.37
Ash	24.24	19.93	38.49
Ca	8.15	7.27	7.37
P	6.75	5.70	6.00
Na	1.70	1.77	1.73

Note: *active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 20 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 20 000 mg; folic acid 400 mg; biotin 40 mg; kobalamin 8.0 mg; choline 100 000 mg; betaine 50 000 mg; Mn 20 000 mg; Zn 16 000 mg; Fe 14 000 mg; Cu 2 400 mg; Co 80 mg; I 200 mg; Se 50 mg.

Table 2 Chemical composition of breast and thigh muscle meat (g.100 g^{-1}).

Parameter	Breast muscle			
	C	P1	P2	P3
Protein	23.42 ±0.21^{b}	23.24 ±0.48^{ab}	22.49 ±0.78^{a}	22.98 ±0.19^{a}
Fat	1.09 ±0.79^{ab}	0.84 ±0.29^{a}	1.96 ±1.06^{b}	1.17 ±0.33^{ab}
Water	74.32 ±0.75	76.13 ±2.51	74.34 ±0.57	74.09 ±0.24
Cholesterol	0.37 ±0.08	0.33 ±0.01	0.39 ±0.06	0.34 ±0.01
	Thigh muscle			
	C	P1	P2	P3
Protein	19.85 ±1.28^{ab}	19.44 ±0.28^{b}	18.70 ±0.29^{a}	19.93 ±0.34^{a}
Fat	7.15 ±1.66^{a}	9.08 ±1.19^{b}	9.15 ±0.14^{b}	7.67 ±2.05^{ab}
Water	71.73 ±1.47^{ab}	70.42 ±0.51^{a}	73.41 ±1.63^{b}	73.34 ±2.60^{b}
Cholesterol	0.70 ±0.05^{a}	0.86 ±0.05^{b}	0.86 ±0.05^{b}	0.78 ±0.15^{ab}

Note: C – control group; P1, P2, P3 – experimental groups; a, b – means with different superscripts within a line differ significantly ($p \leq 0.05$).

muscle between experimental groups P1 and P4 and in thigh muscle between control group C and experimental group P1 and P2.

The lowest average value of fat content measured in fresh breast muscle was in experimental group P1 with addition 0.6 % humic acid (0.84 g.100 g^{-1}) and the higher value was measured in experimental group P2 with addition of humic acid and probiotic (1.96 g.100 g^{-1}). We have found statistically significant differences ($p \leq 0.05$) between experimental groups P1 and P4.

In the case of fresh thigh muscle was measured the higher value of fat content 9.15 g.100 g^{-1} in experimental group P2 with addtion of humic acid and probiotic *Lactobacillus fermentum* and the lowes taverage value in control group C witout addition (7.15 g.100 g^{-1}). We have found statistically significant differences ($p \leq 0.05$) between control group C and experimental group P1 and P2.

The content of fat 2.75 g.100 g^{-1} in breast muscle and 11.98 g.100 g^{-1} in thigh muscle after addition 0.6% humic acids into feeding mixture for broiler chickens Cobb 500 describe in their work **Reitznerová et al. (2016)**.

Ozturk et al. (2010) found content of fat in breast and thigh muscle of broiler chickens Ross 308 after addition 1% humic substances 2.81 g.100 g^{-1} and 11.45 g.100 g^{-1}, respectively. In another work **Ozturk et al. (2011)** reported content of fat in breast and thigh muscle meat of broiler chickens Ross 308 2.67 g.100 g^{-1} in breast muscle and 11.43 g.100 g^{-1} in thigh muscle.

In the case of rabbit meat, **Ondruška et al. (2012**) reported content of fat 1.4 g.100 g^{-1} compared to control group 1.43 g.100 g^{-1} after addition Humac Natur into the feed in amount 0.3% and probiotic.

The water content of fresh breast muscle meat was in control group 74.32 g.100 g^{-1}, while the higher average valuae was measured in experimental group P1 with addition humic acids (76.13 g.100 g^{-1}) and the lowest value in experimental group with addition of coccidiostaticums (P3) 74.09 g.100 g^{-1}. We have not found statistically significant differences ($p \leq 0.05$) between tested groups.

In the thigh muscle was observed the lower value 70.42 g.100 g^{-1} in experimental group P1 and the higher value in experimental group P2 73.41 g.100 g^{-1}. We have found statistically significant differences ($p \leq 0.05$) between experimental group P1 and experimental group P2 and P3.

Ozturk et al. (2010) in their work stated, the content of dry matter in breast muecle of broiler chicken Ross 308 after addition 1% humic substances into fed 26.62 g.100 g^{-1} and in thigh muscle 30.52 g.100 g^{-1} compared to control group 26.86 g.100 g^{-1} and 29.40 g.100 g^{-1}, respectively.

The dry mater content after addition 0.6% humic acids into feed mixture for broiler chickens Cobb 500 was in the work **Reitznerová et al. (2016)** 28.12 g.100 g^{-1} in breast muscle and in the case thigh muscle 33.07 g.100 g^{-1}.

Ozturk et al. (2011) measured content of dry matter in breast muscle of broiler chicken Ross 308 26.55 g.100 g^{-1} and in thigh muscle 30.07 g.100 g^{-1} after addition of 1% humic substances.

In the experiment of **Ondruška et al. (2012)** with addition Humac Natur and probiotic into feed mixture for rabbit reported the total content of water 75.87 g.100 g^{-1} in experimental group and 75.53 g.100 g^{-1} in control group.

The cholesterol content in chicken breasts ranged from 0.33 g.100 g^{-1} (experimental group P1 with addition humic acids) to 0.39 g.100 g^{-1} (experimental group P2 with addition humic acids and probiotic) and in thigh muscle from 0.70 g.100 g^{-1} in control group C to 0.86 g.100 g^{-1} in experimental groups P1 and P2.

We have not found statistically significant differences ($p \leq 0.05$) in breast muscle between tested groups, but in the case of thigh muscle we have found statistically significant differences ($p \leq 0.05$) between control group and experimental group P1 and P2.

Haščík et al. (2016) found cholesterol content in breast muscle of broilers Ross 308 from 86.42 mg.100 g^{-1} in experimental group with propolis addition to 92.17 mg.100 g^{-1} in experimental group with probiotic and in thigh muscle was measured 113.08 mg.100 g^{-1} (probiotic), 118.68 mg.100 g^{-1} (propolis) and in control group 121.25 mg.100 g^{-1}.

Ahmed et al. (2015) found significantly higher crude protein content ($p \leq 0.05$) in the group of broilers fed a diet supplemented with pomegranate in breast muscle (28.55%), as well as thigh muscle (23.44%) than that in non-supplemented group (26.21 and 22.18%, respectively). Moreover, there was a significant decrease ($p \leq 0.05$) in cholesterol content of breast muscle in the pomegranate-supplemented group (62.8 mg.100 g^{-1}) compared with the control (77.44 mg.100 g^{-1}). 63.14 mg.100 g^{-1} (thigh muscle pomegranate) compared with the control (65.78 mg.100 g^{-1}).

CONCLUSION

The results of our study have shown that the application of humic acids or combination of humic acids with probiotics did not effect the chemical composition of breast muscle. In the breast muscle was reducted ($p \leq 0.05$) content of protein in experimental group P3 with coccidiostaticums compared to control group. In thigh muscle was statisticaly higher ($p \leq 0.05$) content of fat and cholesterol in experimental group of chickens fed with Humac Natur or combination Humac Natur and probiotic compared to control group, as confirmed by the results of other authors, who also found a slight increase in fat content and therefore cholesterol in thigh muscle of broiler chicken after using various feed supplements. From a general point of view, we can recommend the application of Humac Natur, or its combination with probiotic into diet of broiler chickens Ross 308.

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