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EFFECT OF FEEDING OF PREFERMENTED BIOPRODUCT CONTAINING GAMMA-LINOLENIC ACID AND BETA-CAROTENE ON SELECTED PARAMETERS OF BROILER CHICKEN MEAT QUALITY

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ABSTRACT

The aim of the work was to evaluate the effect of addition of prefermented bioproduct with a increased content of polyunsaturated fatty acids (especially gamma-linolenic acid) and beta-carotene into commercial feed on the selected qualitative parameters. The chemical composition, the color, the loss of water, the pH and the concentration of lactic acid of the meat of broiler chickens (COBB 500) were monitored. Bioproduct was prepared from corn scrap, which was fermented using the lower filamentous fungus *Umbelopsis isabellina* CCF2412. The prepared material was mixed into the commercial compound feed intended for broiler chickens at a ratio of 10%, and was fed from the 11th day of age of the chickens until the time of slaughter. The obtained results were compared with the results of control group, which was represented by broiler chickens fed only with a commercial compound feed. Feeding of bioproduct, in terms of chemical composition, affected mainly the fat content in breast and thigh meat, which was lower in the experimental group. Meat color (measured by colorimetric assay) was not affected and differences were significant only at a value a*, which was higher in the experimental group. Statistically significant differences in the water losses of meat were not recorded, but the feeding of bioproduct affected the pH of the meat, and also the concentration of lactic acid and both parameters were higher in the meat of control group.

Keywords: bioproduct; gamma-linolenic acid; beta-carotene; broiler chicken; meat quality

INTRODUCTION

A current world trend in human nutrition is the increased demand for human diets containing health beneficial essential polyunsaturated fatty acids (PUFAs) that are not produced by the body and must be obtained through animal feeding. The enrichment of broiler chicken meat with PUFAs is a means of increasing PUFAs consumption in European diets because population intake of broiler chicken meat is high **(Rymer et al., 2010)**. The fatty acid composition of broiler chicken meat can be significantly influenced by feeding **(Zelenka et al., 2008)**, for example by adding a suitable source of PUFAs in the feed **(Zuidhof et al., 2009)**.

Since, PUFAs are one of the main focuses of nutritional-lipid industry; several strategies for their biotechnological production have been developed. One of the promising alternatives of PUFAs production is based on the cultivations of filamentous fungi (order *Mucorales* and *Mortierellales*) in process of solid state fermentation (SSF). The attractiveness of these cultivations is the use of readily available substrates based on waste products from agricultural and industrial production, such as rice bran, wheat bran, oat flakes, and malted draff, peeled or pearled barley **(Čertík and Adamechová, 2009)**. Application of filamentous fungi in SSF process using agricultural waste product and by-products leads to formation of bioproducts enriched with PUFAs and other biologically active compounds, which can be directly used as feed supplements **(Bellou et al., 2016)** to modify the fatty acids profile in poultry **(Bača et al., 2014)**.

However, meat with higher levels of PUFAs has a negative impact on sensory properties and lower nutritional value due to rapid oxidation of the fat. Appropriate system of combination of PUFAs and antioxidants can prevent such losses in meat quality. One possibility is the production of bioproduct using less filamentous fungal strain *Umbelopsis isabellina* CCF2412. Thus prepared organic product is characterized by increased amounts of PUFAs, and in addition also contains beta-carotene, which acts as an antioxidant **(Klempová et al., 2013)**.

There are an insufficient amount of studies dealing with feeding the prefermented bioproduct to broilers and with its effect on meat quality. Therefore, the aim of this study

was to assess effect of feeding of 10% of prefermented bioproduct enriched with γ-linolenic acid (GLA) and contained beta-carotene on the quality indicators of produced meat.

MATERIAL AND METHODOLOGY

The experiment was carried out in accordance with the "European Directive on the the protection of animals used for scientific purposes" **(2010/63/EU)** and with the consent of the State Veterinary and Food Administration of the Slovak Republic no. 3090/13-221 in the premises of Clinic for birds and exotic animals of The University of Veterinary Medicine and Pharmacy in Košice (Slovak Republic).

Animals, diets and management

A total of 80 broiler chickens of meat type hybrid COBB 500 were used in this study. One-day-old chicks were randomly divided into two groups of 40 birds (control and experimental group).

Chickens were reared on deep litter (wood shavings). Temperature and lightning regimes were in accordance with standards for the fattening of broiler chickens **(COBB Broiler Management Guide, 2013)**. During the entire fattening period the broiler chickens had free access to water and feed. Fattening period lasted 39 days and the conventional feed mixtures (Tajba, a.s., Čaňa, Slovak republic), which are used in broiler farms, were fed in both groups. Chickens of the control group were fed only with conventional feed mixtures (Br1, Br2, Br3 and Br4).

Chickens of the experimental group were fed in the same regime and prefermented feed (bioproduct), enriched with GLA and beta-carotene, was administered from the 11th day of fattening. Bioproduct was mixed with the feed in amount of 10% (it means that 10% of conventional feed mixture was replaced by bioproduct).

Clinical health status was continuously monitored. After completion of the fattening period the animals were stunned and killed by cervical dislocation and bled. The carcasses were plucked and eviscerated. Thigh and breast muscles were removed from the carcasses, subsequently packaged in polyethylene bags and stored in a refrigerator at 4 °C until analysis.

Preparation of prefermented bioproduct

Prefermented bioproduct was prepared by the method of fungal solid-state fermentation according to **Čertík et al. (2006)**. Fungal strain *Umbelopsis isabellina* CCF2412 and corn scraps as a substrate were used for preparation of bioproduct. *Umbelopsis isabellina* CCF2412 was obtained from the Culture Collection of Fungi (CCF) (Department of Botany, Charles University, Prague, Czech Republic).

The resulting bioproduct contained in average 3.0 g.kg^{-1} of GLA, and 3.2 mg.kg^{-1} of beta-carotene. Chemical composition of bioproduct, final commercial diet (Br4) and final experimental diet (Br4 +10% bioproduct) is shown in Table 1.

Analysis of muscle

Chemical composition of muscle

Dry matter was determined by oven-drying at 105 °C **(AOAC, 2005)**. Kjeltec Auto, type 1030 analyser (Tecator Co., Hoganas, Sweden) was used to determine the crude protein content. Lipids were isolated in ground samples with petroleum ether in Soxhlet apparatus (LTHS 500, Brnenská Druteva v.d., Czech Republic) and determined gravimetrically.

Color of muscle

The color measurements were carried out using a colorimeter (Chroma meter CR-410, Konica Minolta, Japan) for objective measure of CIE Lab values (L* relative lightness, a* relative redness and b* relative yellowness). Before each measurement, the apparatus was standardized against a white tile. The color values of the breast muscle were measured 24 hours after slaughter and after seven-day storage under chilling conditions (4 °C).

Drip and cook loss of water

For drip loss measurement, breast muscles were packaged in polyethylene bags immediately after deboning, then were weighed and stored in a refrigerator at 4 °C for 24 hours. After 24 hours, samples were weighed again and drip loss was calculated.

Breast and thigh muscles were packed into polyethylene bags and cooked in water bath until internal temperature reached 74 °C. The bags were removed from the water bath and then released liquid was poured off and weighed. Cooking loss was calculated as released liquid × 100/weight before cooking.

Determination of pH

Muscle samples (50 g) were homogenized for 10 min. Then 10 g were used for extraction by distilled water (100 mL) and filtrated. The water extract was used for analysis of pH values by a digital pH meter (inoLab pH720, WTW, Weilheim, Germany) with glass electrode.

Table 1 Chemical composition of bioproduct and final diet (Br4 – control group; Br4 +10% bioproduct – experimental group) used from the 29. day of experiment.

	Bioproduct	Final diet	
		Control group	Experimental group
Crude protein (g.kg^{-1})	102.70	195.90	175.80
Total fat (g.kg^{-1})	80.40	59.40	67.40
Crude fiber (g.kg^{-1})	59.80	46.80	48.10
NDF (g.kg^{-1})	249.10	136.60	148.50
ADF (g.kg^{-1})	85.90	63.20	65.50
ME (MJ.kg^{-1})	12.35	13.41	13.20

Note: NDF = neutral detergent fiber; ADF = acid detergent fiber; ME = metabolized energy.

Table 2 Chemical composition (%) of breast and thigh muscle of broiler chickens.

	Breast muscle		Thigh muscle	
	Control group	**Experimental group**	**Control group**	**Experimental group**
Dry matter	27.9 ±1.4	26.3 ±0.3	32.3 ±1.6*	28.3 ±1.3
Fat	3.5 ±1.0	2.6 ±0.9	12.0 ±2.7	8.3 ±1.4
Total protein	23.3 ±0.1*	22.9 ±0.2	19.9 ±0.7	19.8 ±0.2

Note: n = 10; values in lines (expressed individually for breast and thigh muscle) marked with * are significantly different (* = p <0.05).

Determination of lactic acid

The determination of lactic acid was performed from the water extract that was used for the pH measurements. One ml of water extract was transfered into 10 mL volumetric flasks and filled with distilled water and immediately analyzed by electrophoretic analyser (Type EA102) with a conductive detector (Villa Labeco, Slovak Republic) according to **Mačanga et al. (2011)**. The electrophoretic separation system consisted of a leading electrolyte: 10 mM HCl, β-alanine and 0.1% methylhydroxyethylcellulose (pH 3.2) and terminating electrolyte: 5 mM caproic acid and 5 mM hydroxymethy-aminomethane. The direct currents used in pre-separation and analytical columns were 250 μA and 50μA. The results of analysis were evaluated by the computer programme ITPP pro 32 and expressed in g.100 g^{-1} of muscle.

Statistical evaluation

All the data were analyzed statistically using GraphPad Prism Software, Version 4.00 (Graphpad Prism, 2003). In evaluating the results, Student's t test was used because only two groups (control and experimental) were compared. Statistical significant differences are illustrated in the tables by star marks (* means p <0.05).

RESULTS AND DISCUSSION

Addition of bioproduct produced by solid-state fermentation into commercial broiler feed influenced the chemical composition of the conventional feed mixtures (Table 1) with an impact on the chemical composition of broiler chicken meat (Table 2). Breast and thigh muscles of broiler chickens from the experimental group consisted of slightly lower content of proteins compared to control group. It could be caused by the fact that the diet of the chickens from the experimental group had lower amount of crude protein. On the other hand, experimental diet had higher percentage of fat, but the amount of fat in the muscles of chickens from the experimental group was lower compared to control group. Differences were also recorded in amount of dry matter. Higher values of the dry matter were measured in the muscles of the control group. Statistically more significant differences were found in the thigh muscle (p <0.05).

Similar results of the breast and thigh muscle chemical composition (except percentage of fat) of broiler chickens COBB 500 were described by **Haščík et al. (2011)**. Percentages of fat (breast muscle: 1.28 ±0.26; thigh muscle: 9.40 ±1.23) recorded by these authors are lower than our results.

Based on these results, it has been expected that addition of solid-state prefermented bioproduct into comercial feed mixture had impact not only on chemical composition, mainly amount of fat, but also influence its deposit in organism. However, the higher content of fat was in the feed used in the experimental group, the muscles of this group contained lower percentage of fat, what can be explained by higher deposit of abdominal fat in these chickens. The correctness of this hypothesis could be also confirmed by our results published in the work **Mačanga et al. (2016)**, where is stated, that broiler chickens fed with the bioproduct had on average 37.9 g of abdominal fat compared to chickens of the control group with 29.7 g of abdominal fat.

There are very little information about the effect of feeding product produced by solid-state fermentation on final quality of broiler meat. Comparison of the obtained results with other studies is not possible. However, modification of feedstuff can influence produced broiler meat **(Aziza et al., 2010; Krejči-Treu et al., 2010; Haščík et al. 2012; Bača et al., 2014; Ahmed et al., 2015; Vilarrasa et al., 2015; Elkin et al., 2016)**. The chemical composition of the produced meat, especially fat component was affected by these modifications.

The color of the meat is another parameter that may be influenced by feed. According to results of **Šťastník et al. (2017)** the color of broiler breast muscle was changed after feeding the feed mixtures contained wheats with different grain colour.

Results of our color measurement of breast muscle, expressed in CIE Lab values are presented in Table 3. Addition of prefermented bioproduc tinto the commercial feed mixtures did not affect L* values of breast meat. L* values measured 24 hours after slaughter were almost the

Table 3 The effect of bioproduct on the L*, a* and b* values of broiler breast muscles.

	1st day		7th day	
	Control group	**Experimental group**	**Control group**	**Experimental group**
L*	59.5 ±1.6	59.6 ±2.3	58.6 ±2.0	55.1 ±4.6
a*	11.9 ±1.3	13.5 ±0.6*	10.5 ±1.4	11.5 ±2.4
b*	13.7 ±2.1	11.5 ±1.5	16.1 ±2.4	14.2 ±2.3

Note: n = 10; values in lines (expressed individually for 1st and 7th day) marked with * are significantly different (* = p <0.05).

Table 4 The effect of bioproduct on the drip and cook loss of water (%).

	Drip loss		Cook loss	
	Control group	**Experimental group**	**Control group**	**Experimental group**
Breast muscle	0.5 ±0.1	0.6 ±0.4	33.8 ±9.8	22.4 ±2.5
Thigh muscle	-	-	21.5 ±2.5	22.9 ±0.8

Note: n = 10

Table 5 Values of pH and lactic acid concentration ($g.100g^{-1}$) of breast and thigh muscles.

	Day of the analysis	Breast muscle		Thigh muscle	
		Control group	**Experimental group**	**Control group**	**Experimental group**
pH	**1st day**	5.93 ±0.02*	5.83 ±0.02	6.28 ±0.03*	5.97 ±0.03
	7th day	5.96 ±0.03*	5.88 ±0.01	6.31 ±0.01*	6.11 ±0.02
Lactic acid	**1st day**	1.770 ±0.104	1.332 ±0.285	1.468 ±0.256	1.195 ±0.176
	7th day	1.437 ±0.212	1.535 ±0.207	1.288 ±0.219	1.279 ±0.407

Note: n = 10; values in lines (expressed individually for breast and thigh muscle) marked with * are significantly different (* = p <0.05).

same in the both groups. On the other hand, differences were recorded in a* and b* values. Value a* was significantly higher (p <0.05) in the breast muscle of experimental group and value b* was lower in this group. After 7-day storage in the refrigerator, L*, a* and b* values of the breast muscles packed in the polyethylene bags were changed. In the both groups, L* and a* values slightly decreased and in b* values increasing was recorded. Differences between control and experimental group were almost the same as those found 24 hours after slaughter.

Color of the muscle of broiler chickens is influenced by a lot of factors, such as age, diet, time of storage and so on **(Karaoğlu et al., 2006)**. Authors described that L* value correlates also with pH value of muscle. It means that as pH increases, the L*value decreases. The same correlation was obtained in our work. Differences between control and experimental group in a* and b* values could be caused also by the presence of beta-carotene in the experimental diet.

Drip loss and cook loss of water from muscles were not negatively affected by the bioproduct (Table 4). Drip loss from breast muscles of both groups were almost the equal. After the cooking, loss of water from thigh muscles of control and experimental group were comparable. Cook loss from breast muscles were slightly different, but without statistical significance. Anyway, breast muscles of the control group lost more water after cooking than breast of the experimental group.

By comparing the values of pH (Table 5) in the samples of breast and thigh muscles of both groups, statisticaly significant differences were found (p <0.05). Muscles of the control group had higher pH values 24 hours after slaughter and also after seven-day storage in refrigerator.

Differences were recorded also in the concentrations of lactic acid (Table 5). During first 24 hours after slaughter, higher concentration of lactic acid was measured in the samples of muscles of the control group (p >0.05). During the seven-day storage, the dynamics of lactic acid in the muscles of control and experimental group were different. In the muscles of the control group, decreasing of lactic acid concentration was recorded, while in the muscles of experimental group, lactic acid concentration was increased.

One of the most critical factors affecting the quality of the meat after slaughter is the process of its maturation. The muscles fibres are subjected to biochemical changes called the ripening process, which is an energy-demanding process. The energy is provided by the enzymatic degradation of muscle glycogen to the lactic acid, following decrease of pH in the muscle **(Dalle Zotte, 2002; Čuboň et al., 2004)**. Higher values of lactic acid concentration measured after slaughter in the muscles of control group could mean more intensive breakdown of glycogen in the time before slaughter. But, on the other hand, pH values measured in thsese muscles were not so decreased, and were higher in comparison to experimental group. The pH of muscles is influenced mainly, but not only, by lactic acid concentration. All the metabolic processes in muscles after death are inter-linked and should be considered simultaneously.

Values of pH, L* and loss of water from muscles are correlated. Higher L* values of breast muscle may indicate the qualitative deviation like PSE (pale, soft, exudative) described in pork. **Van Laack et al. (2000)** described that pale breast muscle had L* value 60.0. Value of pH of this muscle was 5.7 and drip loss 1.34%. On the other hand, normal breast muscle had these values: L* 55.1; pH 5.96 and drip loss 0.87%. Simillar values for pale breast muscle are described by **Woelfel et al. (2002),** when L* was 60.4; pH 5.76; drip loss 4.38% and cook loss 26.39%. On the other hand, **Qiao et al. (2002)** described that L* value of normal breast muscles was 62.07 and pH 5.96. Breast muscle marked as lighter-than-normal had L* value 64.34 and pH value 5.82.

L* values obtained in our work could suggest that muscles had qualitative deviation, but on the base of pH values and results of water losses, it is not so.

CONCLUSION

Based on the obtained results, we can conclude that replacing of 10% of the commercial feed by prefermented bioproduct, which is produced from waste of agricultural

production, did not adversely affect the monitored qualitative parameters of broiler chicken meat.

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