Effect of feeding of 5% prefermented cereal-based bioproduct enriched with γ-linolenic acid on production indicators, chemical composition, fatty acid profile and lipid oxidation of broiler meat

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Abstract

The aim of this work was to study the effect of the addition of prefermented cereal-based bioproduct (5%; BP) enriched with γ-linolenic acid to the commercial feed for broiler chickens on the production indicators, chemical composition, fatty acid profile and lipid oxidation of the meat. BP was prepared by solid-state fermentation using fungal strain Cunninghamella elegans CCF 2591. Spelt bran was used as a substrate. A total of 80 broiler chickens (COBB 500) were used in this experiment. Half of them (experimental group) was fed with experimental feed and second half (control group) was fed only with commercial compound feed. Administration of BP to the broilers positively influenced production indicators. Broilers of the experimental group reached higher final weight, and showed lower average daily feed intake, feed conversion ratio, and feed intake compared to the control group. Meat of the experimental group consisted of the lower amount of total protein and fat. BP influenced fatty acid profile as well. Meat of the experimental group, in comparison to control, contained higher amount of unsaturated fatty acids (UFA) and lower amount of saturated fatty acids. Mainly the amount of α-linolenic acid and γ-linolenic acid was higher. Significantly higher concentration of malondialdehyde (MDA) was observed in muscles of experimental group during 7-day storage in refrigerator. Based on the results obtained we can conclude that replacing 5% of the commercial feed by BP could not only improve performance parameters of chickens, but also affect chemical composition and fatty acid content of meat.

Introduction

In recent years, polyunsaturated fatty acids (PUFA) and animal nutrition. PUFA are important for the proper function of the body. The human body is able to synthesise all the fatty acids it needs, except two: linoleic acid and α-linolenic acid. These acids are the main precursors for the synthesis of other fatty acids. They are also called essential fatty acids (Nakamura and Nara 2003). From α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) are synthesised. These n-3 fatty acids have crucial role in anti-inflammatory processes, in diseases such as ischaemic heart disease, depression, aging, cancer and also in autoimmune diseases (Harris et al. 2008). Linoleic acid (LA) and subsequently, γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA) and arachidonic acid (ARA) are important for the synthesis of physiologically active metabolites, prostaglandins (Horrobin 1995). However, the efficiency of these conversions is very poor, especially for n-3 PUFA (Klempová, Mihálík, et al. 2013).

A number of n-3 and n-6 fatty acids can be obtained through diet or supplements (Rubio-Rodríguez et al. 2010). Important sources of essential fatty acids are fish, especially marine fish. Dietary supplementation for example with fish oil, which is rich in PUFA, is reported to have nutritional benefits.
Poultry meat is currently the most preferred, thanks to its low cost, ease of culinary preparation and its dietetic properties. This meat contains higher proportion of unsaturated fatty acids compared to the meat of other slaughtered animals. The fatty acid composition of poultry meat can be significantly influenced by feeding (Zelenka et al. 2008), for example, by adding a suitable source of PUFA into 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 PUFA production is based on the cultivations of filamentous fungi in process of semi-solid state fermentation (SSF). The attractiveness of these cultivations is to use readily available substrates based on waste products from agricultural and industrial production (Čertík et al. 2013).

Filamentous fungi belonging to order Mucorales (Cunninghamella spp., Thamnidium spp., Mucor spp., etc.) and Mortierellales are known as excellent producers of various types of PUFAs. Application of these strains in SSF process using agricultural products and by-products leads to bioproducts enriched with PUFAs and other biologically active compounds. Use of agricultural by-products such as rice bran, wheat bran, oat flakes, and malted draf, peeled or pearled barley in SSF is one of the advantages due to low cost of substrates. Other advantage is that the downstream procedure after SSF in not necessary and bioproducts can be directly used as feed supplements (Bellou et al. 2016) to modify the fatty acid profile in poultry (Bača et al. 2014). Moreover, during SSF fungi produce enzymes necessary for hydrolysis of sources bound in substrate (amyloses, lipases, proteases, etc.) and simultaneously decrease anti-nutrient compounds, which can positively influence not only the animal production parameters (Čertík et al. 2013) but also increase the proportion of n-3 and n-6 fatty acids in the meat (Čertík et al. 2006).

Scantly research has been performed on feeding broilers with prefermented bioproduct and on its effect on growth performance and meat quality. In a previous study, we successfully tested the effect of feeding with 3% of prefermented bioproduct (Bača et al. 2014). Thus, the aim of this study was set to assess effect of feeding with 5% of prefermented cereal-based bioproduct enriched with GLA on broiler growth performance and quality of the meat produced.

**Materials and methods**

**Preparation of prefermented bioproduct**

Prefermented bioproduct was prepared by fungal solid-state fermentation according to Čertík et al. (2006). Fungal strain Cunninghamella elegans CCF 2591 and spelt brans as a substrate were used for preparation of bioproduct. Spelt brans were received from Biomila Company (mill Šajdíkove Humence). Cunninghamella elegans CCF 2591 was obtained from the Culture Collection of Fungi (CCF) of the Department of Botany, Charles University, Prague (Czech Republic). The culture was maintained on potato-dextrose agar slants at 4 °C and was regularly re-inoculated every 3 months. The spore suspension for inoculation was prepared by washing the 7-day old mycelium grown on rice with sterilised distilled water to reach the final concentration of $1 \times 10^6$ spores/mL. Autoclavable microporous high-density polyethylene bags (30 × 40 cm) were filled with 100 g of dry substrate, moistened by the addition of 100 mL of distilled water, soaked for 2 h at laboratory temperature and sterilised in autoclave (105 kPa, 105 °C, 60 min). The substrate was inoculated with 20 mL of the spore suspension (1 $\times 10^6$ spores/mL). The bags were closed and substrate was reincubated at room temperature for 5 days. Prefermented substrate (bioproduct) was then gently oven dried at 65 °C until constant weight was achieved.

**Analysis of feed**

Conventional feed mixtures (HYD 02 and HYD 03), prefermented BP and feed mixtures with 5% of prefermented BP were sampled for analyses. In feed samples, nutrient content was analysed using standard methods and procedures according to the Commission Regulation (EC) no. 691/2013 (Commission Regulation (EC), 2013), which laying down the methods of sampling and analysis for the official control of feed.
Content of dry matter and ash was determined gravimetrically. From the ash, macro- and micro-elements were determined. The content of crude protein was determined by Kjeldahl method. The fat content was determined by Soxhlet extraction, fibre content by two-stage hydrolysis and content of starch was determined polarimetrically. Metabolizable energy values were calculated using regression equations. Fatty acids were determined as their methyl esters by gas chromatography according to Čertík et al. (2006). The gas chromatograph (GC-6890 N, Agilent Technologies, Santa Clara, CA) was equipped with a capillary column DB-23 (0.25 mm, film thickness 0.25 μm, Agilent Technologies, Santa Clara, CA) and a FID detector (constant flow, hydrogen 35 mL/min, air 350 mL/min, 250 °C). Analysis was carried out under a temperature gradient (130 °C–1 min; 130–170 °C–6.5 °C/min; 170–215 °C–2.7 °C/min; 215 °C–7 min; 220–240 °C–2 °C/min; 240 °C–2 min) with hydrogen as a carrier gas (flow 2.1 mL/min, velocity 49 cm/s, pressure 174 kPa) and a split ratio of 1/50 (inlets: heater 230 °C, total hydrogen flow 114 mL/min, pressure 174 kPa). The fatty acid methyl ester peaks were identified by authentic standards for a C4–C24 fatty acid methyl ester mixture (Supelco, Bellefonte, PA) and quantified by an internal standard of heptadecanoic acid (C17:0, Supelco, Bellefonte, PA). The fatty acid concentration was evaluated with ChemStation software B0103 (Agilent Technologies, Santa Clara, CA). All values were means of triplicate determination.

**Animals, diets and management**

The animal protocol for this research was approved by the Ethical Committee for Animal care and Use of University of Veterinary Medicine and Pharmacy in Kosice (Slovak Republic). The experiment was carried out in accordance with the ‘European Directive (Directive 2010/63/EU, 2010) on the protection of vertebrate animals used for experimental and other scientific purposes’ (2010/63/EU) and with the consent of the State Veterinary and Food Administration of the Slovak Republic no. 12492/10-221 in the premises of Clinic for birds and exotic animals of the University of Veterinary Medicine and Pharmacy in Kosice (Slovak Republic).

A total of 80-day-old male broiler chickens of the meat type hybrid COBB 500 were obtained from Hydina Slovensko Broiler Company and were weighed. All the birds were vaccinated at hatching against Newcastle disease and infectious bronchitis. The chicks were randomly divided into two groups of 40 birds (control and experimental groups). Control and experimental groups had four replicates. Each replicate contained 10 birds.

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. Feeding of chickens was carried out as follows: fattening period lasted 35 days and the conventional feed mixtures (purchased from Tajba, a.s., Čaňa, Slovak Republic), which are used in industrial factory farms of broilers, were fed in both the groups. Chickens of the control group were fed only with conventional feed mixtures used in the three phases. At the beginning of fattening, feed mixture ‘HYD 01’ (starter diet) was administered during the first 10 days of fattening. Feeding continued with feed mixture ‘HYD 02’ (growing diet, from 11th to 28th day) and ‘HYD 03’ (the final diet, from 29th to 35th day).

Chickens of the experimental group were fed in the same way, but prefermented bioproduct enriched with GLA was administered from the 15th day of fattening. Bioproduct was added into the feed mixture ‘HYD 02’ and ‘HYD 03’ in amount of 5% (5% of conventional feed mixture was replaced by bioproduct). The compositions of conventional feed mixtures and prefermented bioproduct are shown in Tables 1 and 2.

**Performance and sample collection**

Feed consumption, weight of the animals and clinical health status were continuously monitored. Body weight (BW) of each broiler and feed intake value were recorded at 1st, 14th, 28th and 35th day of age for each replicate. Body weight gains (BWG) and feed conversion ratio (FCR) were calculated from this data. At the end of the fattening period, the average daily gain (ADG), average daily feed intake (ADFI) and FCR were calculated from recorded data.

After completion of the fattening period (35th day) the animals were weighed, stunned, killed by cervical dislocation and bled. The carcasses were plucked, eviscerated (removal of lungs and gastrointestinal tract), and weighed. Thighs with bones and breast muscle was removed from the carcasses and weighed. Thigh and breast muscles were subsequently packaged in polyethylene bags and were stored in a refrigerator at 4 °C until analysis.

Because of carcass yield determination, chickens were weighed before slaughter and after slaughter and subsequent evisceration. Carcass yield was calculated as a ratio of weight of eviscerated carcasses and weight of chickens before slaughter. Thighs with
bones and breast muscle yields were calculated as a percentage of the eviscerated weight.

**Meat quality parameters**

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., Brno, Czech Republic) and were determined gravimetrically. Fatty acid profile was determined by the same method as in the feed.

**Determination of lipid oxidation**

To determine the lipid oxidation changes of thigh and breast muscles, thiobarbituric acid test was used. The extent of lipid oxidation was evaluated as thiobarbituric acid reactive substances (TBARS) by method of Marcinčák et al. (2004). TBARS values were measured spectrophotometrically at 532 nm (Helios γ, v. 4. 6. Thermo spectronic, Cambridge, UK). TBARS values were determined within 24 h after slaughter and after 7-day storage in refrigerator (+4 °C). TBARS results were quantified as malondialdehyde (MDA) equivalents and expressed as mg of malondialdehyde/kg of sample.

**Statistical analysis**

Data were analysed using GraphPad Prism Software, Version 4.00 (Graphpad Prism 2003). The model included the treatment effect, and the cage represented the experimental unit for growth performance parameters, while the average value of the 6 birds per

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### Table 1. Ingredient composition of the broiler diets.

| Ingredients, % | Starter diet | Control | Experimental | Control | Experimental |
|----------------|--------------|---------|--------------|---------|--------------|
| Wheat          | 35.00        | 35.00   | 33.25        | 38.00   | 36.10        |
| Maize          | 31.00        | 23.10   | 21.95        | 22.10   | 21.00        |
| Soybean meal   | 24.50        | 19.10   | 18.15        | 16.50   | 15.68        |
| Sunflower cake | 5.00         | 7.00    | 6.88         | 7.00    | 6.88         |
| Oil            | –            | 1.70    | 1.62         | 2.30    | 2.19         |
| Limestone      | 1.41         | 1.45    | 1.38         | 1.54    | 1.46         |
| Prefermented cereal product | 0.00 | 0.00    | 5.00         | 0.00    | 5.00         |
| Monocalcium phosphate | 1.23 | 1.00    | 0.95         | 0.90    | 0.86         |
| Vitamin-mineral premix | 0.50 | 0.50    | 0.48         | 0.50    | 0.48         |
| L-Lysine       | 0.40         | 0.20    | 0.19         | 0.28    | 0.27         |
| Sodium carbonate | 0.26     | 0.25    | 0.24         | 0.26    | 0.24         |
| NaCl           | 0.25         | 0.25    | 0.24         | 0.26    | 0.24         |
| α-Methionine   | 0.32         | 0.25    | 0.24         | 0.24    | 0.23         |
| L-Threonine    | 0.10         | 0.10    | 0.09         | 0.10    | 0.09         |

*aSupplied per kg of basal diet: vitamin A – 12,500 U; vitamin D₃ – 4000 U; vitamin E – 40 mg; vitamin K₃ – 3 mg; I – 1 mg; Co – 0.7 mg, K – 8.6 g; Cl – 2 g; Cu – 20.0 mg; Fe – 60 mg; Zn – 80 mg; Mn – 90 mg, Se – 0.35 mg.*

### Table 2. Chemical composition of prefermented bioproduct and the broiler diets.

| Ingredients | Bioproduct | Control | Experimental | Control | Experimental |
|-------------|------------|---------|--------------|---------|--------------|
| Dry matter, g/kg | 970.0 | 948.9  | 949.4  | 948.1  | 948.9  |
| Crude protein, g/kg | 208.3 | 212.3 | 205.3 | 198.4 | 200.4 |
| Crude lipids, g/kg | 41.0 | 20.4 | 22.7 | 27.3 | 30.7 |
| Crude fibre, g/kg | 130.5 | 37.1 | 47.3 | 47.3 | 53.4 |
| NDF, g/kg | 460.9 | 122.7 | 140.8 | 125.0 | 151.4 |
| ADF, g/kg | 164.4 | 49.5 | 55.6 | 52.4 | 65.1 |
| Starch, g/kg | 56.1 | 403.3 | 390.7 | 439.3 | 406.7 |
| Ash, g/kg | 104.2 | 60.5 | 62.5 | 59.6 | 63.8 |
| Ca, g/kg | 9.3 | 12.6 | 12.3 | 10.8 | 10.1 |
| Mg, g/kg | 0.2 | 3.3 | 3.4 | 2.8 | 4.1 |
| Na, g/kg | 0.9 | 1.5 | 1.7 | 1.5 | 1.5 |
| K, g/kg | 0.1 | 1.0 | 1.2 | 0.9 | 1.1 |
| P, g/kg | 0.2 | 0.3 | 0.5 | 0.2 | 0.4 |
| Cu, mg/kg | 20.7 | 21.0 | 19.9 | 14.5 | 17.6 |
| Zn, mg/kg | 23.8 | 19.6 | 20.6 | 16.7 | 17.4 |
| Mn, mg/kg | 102.8 | 108.9 | 106.7 | 103.2 | 102.9 |
| ME, MJ/kg | 6.2 | 12.3 | 12.0 | 12.4 | 12.2 |

NDF: neutral detergent fibre; ADF: acid detergent fibre; ME: metabolisable energy.
the treatment was the experimental unit for the carcass yield, chemical composition of the meat, fatty acid and malondialdehyde content. Data are expressed as mean and standard errors of the mean (SEM). Differences between groups were evaluated using t-test with Welch corrections and statistical significance was set at $p < .05$.

**Results and discussion**

Nowadays, majority of nutritional strategies has been designed for the enrichment of poultry meat by PUFA by using linseed oil or vegetable oils or fish oil. However, the use of these oils has resulted in deterioration of the sensory quality of the meat because of the presence of anomalous odours. Both positive effects on fatty acid profile after the usage of these oils, and negative effects of anti-nutritional substances contained in oils on poultry growth parameters were observed (Aziza et al. 2010; Pietras and Orczewska-Dudek 2013). Application of prefermented cereal BP, observed (Aziza et al. 2010; Pietras and Orczewska-Dudek 2013). Employment of prefermented cereal BP, with a higher content of PUFA, has the potential to prevent mentioned problems as it has a reduced content of anti-nutritional compounds compared to the traditional plant materials. Since the PUFA-oil is present in BP, it is protected from oxidation that results in unpleasant smell of oil thanks to the antioxidants contained in cereals. A conventional feed supplies mainly n-6 PUFA and a small amount of n-3 PUFA. This is reflected in the fatty acid composition of meat produced with an increased content of n-6 fatty acid, mainly LA. Modifications in the fatty acid composition of poultry meat require addition of the PUFA source to feed. A current global trend in the production of diets with supplemented components of PUFA has increased the demand for feedings containing GLA in animal nutrition (Laho et al. 2011). However, GLA is rarely found in standard feed. Therefore, interest has been focussed on alternative sources of GLA. Oleaginous lower filamentous fungi have been known as a good source of GLA-rich oil (Certik et al. 2013). Employment of these fungi in SSF process leads to bioproduct enriched with GLA that can be directly used as supplement for poultry diet.

In our experiment, bioproduct produced by fungal SSF positively affected the conventional feed mixtures (Table 2). Amount of total protein was increased, mainly in experimental final diet. In experimental grower diet, concentration of total protein was slightly reduced because conventional growing feed mixture contained higher percentage of proteins compared to bioproduct. Concentration of the fat was higher in both experimental diets compared to conventional feed mixtures. Spelt beers used for BP preparation are rich in fibre content that was even more elevated after fermentation. Therefore, supplemented BP increased the fibre content in experimental diets, that had slightly lower values of metabolisable energy and concentration of some macro- and micro-elements.

Application of 5% BP into both growing and final feed mixtures changed profile of fatty acids as well (Table 3). Fungal strain is able to utilise LA in cereal for GLA biosynthesis. Analysis of spelt bran before/after SSF is in consistency with previous data. The content of LA in total fatty acids (TFA) during SSF drops from 55.51 to 42.80%. Prefermented BP contains 8.45% of GLA in TFA (2.45 g GLA/kg BP). The most significant differences in the proportions of fatty acids in conventional and experimental feed mixtures were reported in concentration of LA and GLA. Concentration of GLA in TFA increased from zero (conventional feed mixture HYD 02 and HYD 03) to 0.57% (experimental diets). On the other hand, concentration of LA in TFA was decreased.

The effect of addition of 5% of prefermented BP into commercial feed on growth performance is shown in Table 4. BP was administered from the 15th day of fattening, therefore, no significant differences were observed in growth performance between control and experimental groups from 1st to 14th day. Significant differences ($p < .05$) in body weight, feed consumption, weight gain and FCR were detected in the period from 15th to 28th and also from 29th to 35th day of age. After 35 days of rearing, birds reached live body weight ranging from $2122 \pm 194$ g in control group to $2234 \pm 189$ g in experimental group. The experimental group had higher final weight ($p < .05$), while broiler chickens of this group consumed significantly ($p < .05$)

| Table 3. Fatty acid profile of prefermented bioproduct and the broiler diets ($n = 6$ replicates/diet). |
| --- | --- | --- | --- |
| Fatty acid, % | Bioproduct | Control | Experimental | Control | Experimental |
| C14:0 | 0.52 | 0.00 | 0.02 | 0.00 | 0.02 |
| C16:0 | 13.91 | 13.58 | 13.19 | 12.83 | 12.42 |
| C16:1n-9 | 0.37 | 0.00 | 0.09 | 0.00 | 0.08 |
| C16:1n-7 | 0.53 | 0.18 | 0.17 | 0.14 | 0.15 |
| C18:0 | 3.01 | 2.50 | 2.38 | 2.46 | 2.45 |
| C18:1n-9 | 25.48 | 21.77 | 22.76 | 22.52 | 22.84 |
| C18:2n-6 | 4.20 | 56.55 | 55.59 | 56.60 | 55.16 |
| C18:3n-6 | 8.45 | 0.00 | 0.57 | 0.00 | 0.57 |
| C18:3n-3 | 1.86 | 3.39 | 3.17 | 3.44 | 3.40 |
| C20:0 | 0.30 | 0.37 | 0.34 | 0.36 | 0.36 |
| C20:1n-9 | 0.89 | 0.31 | 0.38 | 0.28 | 0.30 |
| C22:0 | 0.31 | 0.24 | 0.24 | 0.25 | 0.24 |
| C24:0 | 0.79 | 0.20 | 0.23 | 0.17 | 0.18 |
| $\sum$ SFA | 18.84 | 16.89 | 16.39 | 16.06 | 15.68 |
| $\sum$ MUFA | 28.05 | 23.16 | 24.28 | 23.90 | 24.22 |
| $\sum$ PUFA | 53.11 | 59.95 | 59.33 | 60.04 | 60.12 |

$\sum$ SFA: sum of saturated fatty acids; $\sum$ MUFA: sum of monounsaturated fatty acids; $\sum$ PUFA: sum of polyunsaturated fatty acids.
smaller amount of feed (3208 g), compared to the control group (3595 g). The experimental group, in comparison with control group, showed a lower ADFI and FCR (p < .05).

This paper presents an initial study of the use of prefermented feed (after fermentation by Cunninghamella elegans) in the diet of broiler chickens to increase the proportion of GLA in produced meat. Due to scanty data available in this field, we cannot compare the results obtained here with studies from other researchers. The comparable results are published only in our previous study (Baća et al. 2014) where supplementation of commercial feed with 3% of the prefermented BP (produced by Thamnidium elegans fermentation) had also positive effect on the production indicators. Broilers fed with experimental diets reached higher final weight, while feed intake was lower. Fungal strain employed in SSF process has the ability to produce various types of hydrolytic enzymes (amylases, glucanases, etc.) that are necessary to cleave biopolymers in cereals. Products of these reactions are usually mono- or disaccharides, which serves as carbon source for microorganism. However, after fermentation these enzymes are still present in BP and in experimental feed mixture. The positive effects of exogenous enzymes in poultry diets are widely known and represent one of the trends in modern poultry science (Ravindran 2013; Vieira et al. 2014). On the other hand, in the studies where the oils, seeds or oil rich plants were used to supplement PUFA for birds, changes were not recorded (Qi et al. 2010) or production indicators were regressed (Sirri et al. 2003; Aziza et al. 2010; Jaskiewicz et al. 2014). Sirri et al. (2003) fed broilers (COBB 500) with diets supplemented with 2 or 4% conjugated linoleic acid. After 47 day of rearing, body weight, daily weight gain and feed intake have been not influenced by dietary treatment of birds.

Similar positive effect on growth parameters of broilers was recorded in the study performed by Chu et al. (2017) after feeding with fermented wheat bran. The wheat brans were fermented by Trichoderma pseudokoningii. Trichoderma species are known for producing cell well-degrading enzymes (cellulase, xylanase). Replacement of 10% of the basic diet by the fermented wheat brans resulted in decreasing of feed consumption and improvement of FCR in the experimental chickens.

For feed supplementation of broilers Aziza et al. (2010) used non-fermented Camelina (Camelina sativa) meal as a rich source of n-3 fatty acids (mainly ALA). Camelina belongs to the Brassica family, and species of this family are high in nonstarch polysaccharides and glucosinolates, which can affect feed consumption and bird performance. In the mentioned work, chickens of the experimental groups were fed basal diet with added Camelina meal at 2.5, 5 and 10% for 42 day. No significant differences in final weight, BWG and FCR were observed among birds of the control and experimental diets. The use of Camelina sativa (raw seeds, meal or expellers) up to 10% of the diet resulted in a deterioration of the growth performance of chicken (Pekel et al. 2009; Jaskiewicz et al. 2014).

Feeding broiler chickens with feed containing prefermented BP in amount of 5% had significant effect on carcase eviscerated yield, but thigh and breast muscle yields were not affected (Table 5).

The chemical composition of the breast and thigh muscles of chickens of control and experimental groups is shown in Table 6. According to the results obtained it is evident that prefermented BP significantly affected the chemical composition of meat. Moisture in the meat of the experimental group was

### Table 4. Effect of of prefermented bioproduct supplemented in diet on growth performance of 1–35 d-old broilers.

| Item                  | Control | Experimental | SEM   | p value |
|-----------------------|---------|--------------|-------|---------|
| 1–14 days             |         |              |       |         |
| Body weight (g)       | 432     | 436          | 7.8   | .082    |
| Feed consumption (g)  | 467     | 464          | 4.5   | .070    |
| Weight gain (g)       | 395     | 399          | 8.9   | .320    |
| FCR                   | 1.19    | 1.16         | .01   | .201    |
| 15–28 days            |         |              |       |         |
| Body weight (g)       | 1544    | 1599         | 17.5  | .020    |
| Feed consumption (g)  | 1485    | 1345         | 19.7  | .001    |
| Weight gain (g)       | 1112    | 1163         | 11.9  | .005    |
| FCR                   | 1.29    | 1.16         | .01   | .002    |
| 29–35 days            |         |              |       |         |
| Body weight (g)       | 2122    | 2234         | 41.6  | .013    |
| Feed consumption (g)  | 1643    | 1399         | 19.5  | .001    |
| Weight gain (g)       | 578     | 635          | 10.8  | .003    |
| FCR                   | 2.84    | 2.36         | .04   | .002    |
| 1–35 days             |         |              |       |         |
| Feed consumption (g)  | 3595    | 3208         | 11.2  | .001    |
| Weight gain (g)       | 2085    | 2197         | 48.3  | .055    |
| FCR                   | 1.72    | 1.46         | .02   | .001    |
| ADG, g                | 60      | 63           | 1.0   | .051    |
| ADFI, g               | 103     | 91           | 1.7   | .002    |

The results are provided as the means of four replicates (10 birds/repli- cate) in each control and experimental group (n = 4). SEM: standard error of the mean.

Means in the same lines with different superscripts are significantly different (p < .05).

ADFI: Average daily feed intake; ADG: average daily gain; FCR: feed conversion ratio.

### Table 5. Effect of prefermented bioproduct on carcase yield of broiler chickens.

| Item                  | Control | Experimental | SEM   | p value |
|-----------------------|---------|--------------|-------|---------|
| Yields, %             |         |              |       |         |
| Eviscerated carcass    | 72.38   | 74.15        | 0.14  | .001    |
| Breast muscles        | 24.13   | 24.25        | 0.10  | .226    |
| Thigh with bones      | 29.23   | 29.45        | 0.10  | .086    |

The results are provided as the means of four replicates (10 birds/repli- cate) in each control and experimental group (n = 4). SEM: standard error of the mean.

Means in the same lines with different superscripts are significantly different (p < .05).
higher \((p < .05)\). Samples of breast and thigh muscle of experimental group had reduced proteins content compared to control \((p < .05)\), and also the fat content was significantly lower \((p < .05)\).

Fatty acid content of the breast and thigh muscles is shown in Table 7. Addition of prefermented BP into the broilers feed had effect on the profile of fatty acids of the meat. The amount of saturated fatty acids was lower in the meat of experimental group, mainly in the breast muscle \((p < .05)\), because of the decline of palmitic acid and stearic acid. The total amount of unsaturated fatty acids was influenced by BP supplementation \((p < .05)\) only in the breast muscle. No significant differences were observed in content of MUFA and also the total amount of PUFA was not significantly changed. On the other hand, significantly higher amount of GLA, ALA and stearidonic acid \((18:4n-3, SDA)\) was recorded in the meat of experimental group.

As we expected, GLA acid content increased in both muscles of the experimental group broiler chickens \((p < .05)\). Increased concentration of GLA in meat was not proportional, because of the increased GLA concentration in the experimental feed \((0.57\%)\), a higher amount of GLA in the meat was expected. A lower proportion of GLA in meat compared to the feed could also be affected by the length of fattening. In our case, the feeding of BP took only 20 days, which may be insufficient to increase significantly the proportion of fatty acids in chickens’ meat. Jaskiewicz et al. (2014) indicated that the proportion of ALA in breast muscle fat after feeding *Camelina sativa* oil, soybean and rapeseed oils increases with the extended period of feeding and with increased doses of fatty acids in feed. Based on our results, we can conclude that the fatty acid composition of feed influences the fatty acid composition of meat. Similarly, other authors (Narciso-Gaytán et al. 2011; Tres et al. 2014) reported that fatty acid composition of chicken muscles reflected the fatty acid composition of the dietary oils.

In the breast and thigh muscles of both groups, fatty acids were represented mostly by palmitic acid, oleic acid and LA, and this composition was related to fatty acid composition of the dietary oils. Based on our results, we can conclude that fatty acid composition of meat is correlated with fatty acid composition of the conventional feed mixture, base of the feed of both groups.

Most authors used linseed oil (Lopez-Ferrer et al. 2001; Zelenka et al. 2008; Pietras and Orczewska-Dudek 2013), Chia seed (Ayerza et al. 2002) or olive oil (Crespo and Esteve-Garcia 2002) to increase PUFA in meat.

### Table 6. Chemical composition (%) of breast and thigh muscle of broiler chickens \((n = 6\) replicates/treatment).

|                | Control | Experimental | SEM  | \(p\) value |
|----------------|---------|--------------|------|-------------|
| **Breast muscle** |         |              |      |             |
| Moisture       | 73.03\(^a\) | 75.27\(^b\) | 0.19 | <.001       |
| Fat            | 4.11\(^a\)  | 2.45\(^b\)  | 0.02 | <.001       |
| Total protein  | 21.65\(^a\) | 20.85\(^b\) | 0.02 | <.001       |
| **Thigh muscle** |         |              |      |             |
| Moisture       | 70.31\(^b\) | 73.37\(^a\) | 0.27 | <.001       |
| Fat            | 9.32\(^a\)  | 6.50\(^b\)  | 0.14 | <.001       |
| Total protein  | 19.44\(^a\) | 17.91\(^b\) | 0.18 | <.001       |

SEM: standard error of the mean.

Means in the same lines with different superscripts are significantly different.

### Table 7. Fatty acids content (g per 100 g of total fat) of breast and thigh muscle of broiler chickens \((n = 6\) replicates/treatment).

| Fatty acid  | C       | E       | SEM | \(p\) value | C       | E       | SEM | \(p\) value |
|-------------|---------|---------|-----|-------------|---------|---------|-----|-------------|
| **Breast muscle** |         |         |     |             |         |         |     |             |
| C14:0       | 0.36    | 0.39    | 0.01| .151        | 0.37    | 0.37    | 0.01| .432        |
| C16:0       | 18.12   | 17.83   | 0.32| .126        | 21.32   | 20.97   | 0.68| .009        |
| C16:1n-7    | 3.13    | 3.78    | 0.15| .069        | 4.63    | 4.31    | 0.21| .207        |
| C18:0       | 8.27    | 7.76    | 0.38| .247        | 8.38    | 8.14    | 0.15| .230        |
| C18:1n-9    | 23.33   | 23.98   | 1.15| .401        | 26.14   | 26.68   | 0.33| .196        |
| C18:2n-6    | 4.08    | 3.72    | 0.35| .289        | 3.04    | 2.84    | 0.19| .279        |
| C18:3n-6    | 15.43   | 16.32   | 0.39| .154        | 17.03   | 17.12   | 0.28| .428        |
| C18:3n-9    | 0.09\(^b\) | 0.16\(^a\) | 0.01| .008        | 0.11\(^b\) | 0.19\(^a\) | 0.01| .013        |
| C18:3n-6    | 0.44\(^b\) | 0.55\(^a\) | 0.01| .018        | 0.56\(^b\) | 0.69\(^a\) | 0.02| .046        |
| C18:4n-3    | 0.14\(^a\) | 0.20\(^a\) | 0.01| .014        | 0.04\(^a\) | 0.17\(^a\) | 0.01| .010        |
| C20:1n-9    | 0.48\(^b\) | 0.36\(^a\) | 0.03| .033        | 0.36    | 0.35    | 0.02| .002        |
| C20:2n-6    | 0.85    | 0.70    | 0.35| .187        | 0.33    | 0.37    | 0.01| .051        |
| C20:3n-6    | 1.17    | 1.22    | 0.12| .416        | 0.59    | 0.58    | 0.05| .443        |
| C20:4n-6    | 4.16    | 4.16    | 0.29| .496        | 3.64    | 3.55    | 0.26| .423        |
| C20:5n-3    | 0.29    | 0.26    | 0.03| .277        | 0.14    | 0.15    | 0.01| .339        |
| C22:5n-3    | 0.39    | 0.50    | 0.03| .173        | 0.39    | 0.43    | 0.02| .161        |
| C22:6n-3    | 0.40    | 0.41    | 0.02| .455        | 0.21    | 0.25    | 0.01| .091        |
| \(\sum\) SFA | 29.97\(^a\) | 27.82\(^b\) | 0.43| .046        | 27.71   | 26.44   | 0.46| .077        |
| \(\sum\) UFA | 55.03\(^b\) | 57.18\(^a\) | 0.43| .046        | 57.29   | 58.56   | 0.46| .077        |
| \(\sum\) PUFA, n-3 | 1.86    | 1.81    | 0.07| .366        | 1.38    | 1.59    | 0.06| .083        |
| \(\sum\) PUFA, n-6 | 20.86   | 22.04   | 0.65| .199        | 21.25   | 21.96   | 0.38| .193        |
| n-6/n-3 ratio | 11.25   | 12.02   | 0.31| .095        | 15.40   | 13.83   | 0.45| .055        |

C: control; E: experimental. \(\sum\) SFA: sum of saturated fatty acids; \(\sum\) UFA: sum of unsaturated fatty acids; \(\sum\) PUFA: sum of polyunsaturated fatty acids.

Within criterion, means in the same lines with different superscripts are significantly different \((p < .05)\).
poultry meat. In the cited studies, the administered content of n-3 and n-6 PUFA was similar. These administrations resulted in the muscles with increased content of fatty acids, represented by ALA, the main fatty acid in the served feed.

In our work, an increase content of GLA in meat responded to composition of feed with BP. This means that the feeding of the cereal prefermented BP resulted in the increasing of the desired GLA in broilers muscles. Although this fatty acid belongs to the group of n-6 fatty acids, it has significant anti-inflammatory role, affects the progress of sclerosis multiplex and serves as treatment for atopic eczema (Simon et al. 2014).

Feeding broilers with diets containing prefermented BP changed the percentage of ALA, GLA and SDA in broiler meat of the experimental group, which was also reflected in the degradation changes of fats (Table 8). Regarding the lipid oxidation stability of the meat, the results indicate that as the proportion of UFA in chicken increases, the susceptibility of meat to lipid oxidation also increases over the storage time (Narciso-Gaytán et al. 2011). The amount of MDA was increased in both muscles of both groups during 7-day storage in refrigerator (p < .05). Meat samples from broilers fed with BP were more susceptible to oxidation compared to control. The higher lipid content of thigh meat compared with breast meat is reflected in its lower oxidative stability because MDA content was more concentrated in the thigh meat (Rymer et al. 2010). Significantly higher increase of MDA concentration was observed in muscles of experimental group, which is caused by higher amount of PUFA in these muscles. Even though meat oxidation stability was lower, the amount of MDA was not high enough to negatively affect the quality of meat.

The enhancement of chicken meat with unsaturated fatty acids has been shown to reduce oxidation stability of the meat. The most strategies used to influence the fatty acid profile of broiler meat also require the addition of antioxidants to reduce the process of oxidation of lipids and thus to preserve the quality and shelf life of meat (Rymer et al. 2010; Jaskiewicz et al. 2014). Tres et al. (2014) concluded that the tissue susceptibility to oxidation is dependent on their PUFA/α-tocopherol ratios. The α-tocopherol is an effective antioxidant in animal nutrition. Similarly, Nkukwana et al. (2014) reported that the oxidative stability of poultry meat was higher after feeding oil extracted from the Moringa oleifera leaves, as this oil is a major source of natural antioxidants (mainly flavonoids).

Conclusions
Based on the obtained results we can conclude that replacing 5% of the commercial feed by BP, which is produced from the waste of agricultural production, positively improved the monitored performance parameters of broiler chickens and affected chemical composition and fatty acid content of produced meat.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This study was supported by The Slovak Research and Development Agency under the contracts APVV-14-0397 and APVV-0662-11 and by the Ministry of Education of the Slovak Republic (Project VEGA No. 1/0457/14 and VEGA No. 1/0574/15).

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Table 8. Effect of prefermented bioproduct on the amount of malondialdehyde (mg/kg) in muscle samples during storage (4°C) (n = 6 replicates/treatment).

| Day of storage | Control | Experimental | SEM | p value |
|---------------|---------|--------------|-----|---------|
| Breast muscle |         |              |     |         |
| 1st           | 0.141   | 0.147        | 0.006 | .286   |
| 7th           | 0.280a  | 0.328b       | 0.010 | .019   |
| Thigh muscle  |         |              |     |         |
| 1st           | 0.154   | 0.157        | 0.006 | .365   |
| 7th           | 0.242a  | 0.287b       | 0.012 | .027   |

SEM: standard error of the mean.
Means in the same lines with different superscripts are significantly different (p < .05).
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