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Fatty acids profile of breast and thigh muscles of broiler chickens fed diets with propolis and probiotics

Obsah mastných kyselín v prsnej a stehennej svalovine brojlerových kurčiat kŕmených s prídavkom propolisu a probiotík

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Abstract

The aim of the study was to assess the effect of supplying propolis extract separately and propolis extract together with probiotics based on *Lactobacillus fermentum* on fatty acids (FA) composition of the most valuable parts of chicken carcass. Diets enriched with 400 mg propolis extract per 1 kg of feed mixture and 400 mg propolis extract per 1 kg of feed mixture plus 3.3 g probiotic preparation added to drinking water (E1 and E2 groups, respectively) were given to broiler chickens throughout a 42-d growth period. After slaughter, the FA profiles of breast and thigh samples were determined. Both supplemented diets decreased the total amount of saturated FA (SFA), mainly because of the myristic (C14:0) and stearic (C18:0) acid contents in both breast and thigh muscles. However, a significant decrease ($P \leq 0.05$) in SFA was confirmed only in thigh muscle. Supplementation with propolis together with probiotics (E2) significantly increased ($P \leq 0.05$) monounsaturated FA (MUFA) contents in breast muscle. Particularly oleic acid (C18:1 cis) contributed to an overall increase in MUFA. A rise ($P \leq 0.05$) in polyunsaturated FA (PUFA) in breast muscle was, however, associated with the dietary supplementation of propolis extract separately (E1). A similar trend ($P > 0.05$) for MUFA and PUFA levels was also observed in thigh muscle. Of all PUFAs detected in breast and thigh muscles, linoleic acid (C18:2 cis) was found at the highest levels. Its levels varied from 11.34 to 12.02 g*100 g^{-1} and from 11.05 to 11.82 g*100 g^{-1} in breast and thigh muscles, respectively. The highest level ($P \leq 0.05$) of linoleic acid was observed in group E1. Comparing breast with thigh muscle, the breast was demonstrated to contain more SFA and PUFA, but less MUFA proportions. Although the n-3 PUFA:n-6 PUFA ratio was similar among the treatments in both breast and thigh muscles, n-6 PUFA:n-3 PUFA ratio has been showed to be a significantly lower ($P \leq 0.05$) in thigh muscle,

with the lowest ratio found in E2 group. Differences in n-6 PUFA:n-3 PUFA ratio in breast were, however, not significant ($P > 0.05$) between the treatments. On the whole, the present study indicated that propolis applied through the feed either separately or together with probiotics applied through the water had favourable effect on fatty acids profile in the most valuable parts of chicken carcass and could be thus added to diets for broiler chickens.

Keywords: breast, broiler chicken, fatty acid, probiotic, propolis, thigh

Abstrakt

Cieľom štúdie bolo posúdiť vplyv propolisového extraktu aplikovaného samostatne alebo v kombinácii s probiotikom na báze *Lactobacillus fermentum* na obsah mastných kyselín v najcennejších častiach jatočného tela kurčiat. Počas 42 dní výkrmu bol kurčatám podávaný propolisový extrakt v množstve 400 mg na 1 kg kŕmnej zmesi (skupina E1) a propolisový extrakt v množstve 400 mg na 1 kg kŕmnej zmesi spolu s 3,3 g probiotického preparátu pridávaného do pitnej vody (skupina E2). Po porážke kurčiat bol stanovený obsah mastných kyselín vo vzorkách prsnej a stehennej svaloviny. Aplikáciou preverovaných doplnkov došlo k zníženiu celkového množstva nasýtených mastných kyselín (SFA), a to hlavne pre zníženie obsahu kyseliny myristovej (C14:0) a stearovej (C18:0) v prsnej aj v stehennej svalovine. Štatisticky preukazný rozdiel ($P \leq 0,05$) bol však zaznamenaný len v stehennej svalovine. Obsah mononenasýtených mastných kyselín (MUFA) bol zvýšený ($P \leq 0,05$) po aplikácii propolisového extraktu spolu s probiotikami (E2), a to v prsnej svalovine. K celkovému zvýšeniu MUFA prispel najmä vysoký obsah kyseliny olejovej (C18:1 cis). Obsah polynenasýtených mastných kyselín (PUFA) v prsnej svalovine bol najvyšší ($P \leq 0,05$) v skupine s prídavkom propolisového extraktu aplikovaného samostatne (E1). V stehennej svalovine bol v obsahu MUFA a PUFA pozorovaný podobný trend ako v prsnej svalovine, ale bez významných rozdielov medzi skupinami ($P > 0,05$). V rámci PUFA detegovaných v prsnej a stehennej svalovine bol zistený najvyšší obsah kyseliny linolovej (C18:2 cis), a to od 11,34 do 12,02 g*100 g^{-1} v prsnej a od 11,05 do 11,82 g*100 g^{-1} v stehennej svalovine. Najvyššie množstvo ($P \leq 0,05$) kyseliny linolovej bolo v oboch svaloch zistené v skupine E1. Celkovo sa prsná svalovina vyznačovala vyšším zastúpením SFA a PUFA, a naopak nižším podielom MUFA v porovnaní so stehennou svalovinou. Pomer n-3 PUFA:n-6 PUFA bol vo všetkých skupinách približne na rovnakej úrovni v prsnej aj v stehennej svalovine. Pomer n-6 PUFA:n-3 PUFA bol však nižší ($P \leq 0,05$) v stehennej svalovine kurčiat z pokusných skupín, pričom najnižšia hodnota bola zaznamenaná v skupine E2. V prsnej svalovine neboli významné rozdiely ($P > 0,05$) v pomere n-6 PUFA:n-3 PUFA. Na záver možno konštatovať, že prídavok propolisového extraktu do krmiva samostatne alebo v kombinácii s probiotikom aplikovaným cez vodný zdroj mal priaznivý vplyv na profil mastných kyselín v najcennejších svaloch kuracieho mäsa, a preto je vhodným kŕmnym doplnkom vo výžive brojlerových kurčiat.

Kľúčové slová: brojlerové kurča, mastná kyselina, probiotikum, propolis, prsia, stehná

Detailný abstrakt

Cieľom experimentu bolo skúmanie vplyvu propolisového extraktu a jeho kombinácie s probiotikom na báze *Lactobacillus fermentum* ($1*10^9$ KTJ v 1 g nosného média) na obsah mastných kyselín v najcennejších častiach jatočného tela kurčiat. Do experimentu bolo zahrnutých 180 ks jednodňových kurčiat hybridnej kombinácie Ross 308, ktoré boli rozdelené na tri skupiny: 1. kontrolná skupina – bez prídavku propolisového extraktu a probiotika (C), 2. pokusná skupina – prídavok propolisového extraktu v množstve 400 $mg*kg^{-1}$ kŕmnej zmesi (E1) a 3. pokusná skupina – prídavok propolisového extraktu v množstve 400 $mg*kg^{-1}$ kŕmnej zmesi a probiotického preparátu v množstve 3,3 g pridávaného denne do pitnej vody (E2). Výkrm kurčiat trval 42 dní a bol realizovaný na hlbokej podstielke. Kŕmenie bolo rozdelené na dve fázy: 1. štartérová (vek 1 až 21 dní, kŕmna zmes HYD-01 v sypkej forme) a 2. rastová (vek 22 až 42 dní, kŕmna zmes HYD-02 v sypkej forme). Kŕmne zmesi neobsahovali antibiotiká ani kokcidiostatiká. Príjem krmiva a vody bol zabezpečený systémom *ad libitum*. Po skončení výkrmu bol v prsnej a stehennej svalovine stanovený obsah mastných kyselín. Prsná ($P > 0,05$) aj stehenná svalovina ($P \leq 0,05$) kurčiat z pokusných skupín obsahovala nižšie množstvo nasýtených mastných kyselín (SFA), na čom sa podieľal najmä nižší obsah kyseliny myristovej (C14:0) a stearovej (C18:0). Významné zvýšenie ($P \leq 0,05$) sa prejavilo v obsahu mononenasýtených mastných kyselín (MUFA) v prsnej svalovine v skupine E2. K zvýšeniu celkového obsahu MUFA v skupine E2 prispel najmä vyšší obsah kyseliny olejovej (C18:1 cis). Celkový obsah polynenasýtených mastných kyselín (PUFA) bol v prsnej svalovine najvyšší ($P \leq 0,05$) v skupine E1. Množstvo MUFA a PUFA malo v skupinách experimentu podobnú tendenciu aj v stehennej svalovine ($P > 0,05$). Z PUFA bol detegovaný najvyšší obsah kyseliny linolovej (C18:2 cis) v prsnej (11,34 – 12,02 $g*100\ g^{-1}$) a v stehennej svalovine (11,05 – 11,82$*100\ g^{-1}$). Najvyšší obsah ($P \leq 0,05$) kyseliny linolovej v oboch svaloch bol v skupine E1. Výsledky potvrdili, že v prsnej svalovine boli SFA a PUFA obsiahnuté vo vyššom a MUFA v nižšom množstve ako v stehennej svalovine. Pomer n-3 PUFA:n-6 PUFA bol vo všetkých skupinách experimentu na približne rovnakej úrovni. Avšak, pomer n-6 PUFA:n-3 PUFA bol v stehennej svalovine nižší ($P \leq 0,05$) v pokusných skupinách, pričom najnižšia hodnota bola zaznamenaná v skupine E2. V prsnej svalovine neboli významné rozdiely ($P > 0,05$) v pomere n-6 PUFA:n-3 PUFA. Získané výsledky preukázali pozitívny vplyv propolisového extraktu aplikovaného vo výžive brojlerových kurčiat cez kŕmne zmesi samostatne alebo v kombinácii s probiotikom aplikovaným cez vodný zdroj na zloženie mastných kyselín v prsnej a stehennej svalovine, vzhľadom na priaznivý obsah viacerých mastných kyselín nevyhnutných pre správnu výživu ľudí. Účinky preverovaných doplnkov však neboli jednoznačne potvrdené vo všetkých mastných kyselinách, preto odporúčame ich ďalšie preskúmanie s cieľom overiť ich účinnosť.

Introduction

Poultry meat has many desirable nutritional characteristics, such as high protein content, low lipid content and relatively high concentrations of essential fatty acids (Gallardo et al., 2012; Nkukwana et al., 2014). In recent years, there has been interest in the role of poultry meat as a dietary source of long chain n-3 polyunsaturated fatty acids (PUFA), including alpha-linolenic acid (ALA, 18:3), eicosapentaenoic acid (EPA, 20:3) and docosahexaenoic acid (DHA, 22:6) (Dalziel et al., 2015).

Adequate intake of n-3 fatty acids (FA), a balanced n-3 PUFA/n-6 PUFA ratio, even a proper polyunsaturated FA/saturated FA ratio (PUFA/SFA), may reduce the risk of life-style diseases such as coronary artery disease, hypertension, diabetes, and inflammatory and immune disorders (Zhou et al., 2012). There is evidence suggesting that saturated fatty acids (SFA) have negative consequences on human health whereas polyunsaturated fatty acids (PUFA) have beneficial effects (Gibbs et al. 2013); PUFA should constitute 7% of total energy consumed (Soriano-Santos et al., 2010).

Nutritionists have also focused on the type of PUFA and the balance in the diet between n-3 PUFA formed from α-linolenic acid (18:3) and n-6 PUFA formed from linoleic acid (18:2) (Wood et al., 2003), and recommend to increase the intake of n-3 PUFA at the expense of n-6 PUFA (Mapiye et al., 2011; Parunović et al., 2012).

There are two reasons for the increasing level of FA polyunsaturation in chicken meat. First, human nutritionists recommend reducing the intake of saturated fatty acids (SFA) because of its relationship with the development of cardiovascular diseases. Second, the use of animal fats has been reduced in Europe, in favour of vegetable oils that are more polyunsaturated (Cortinas et al., 2004). According to Morales-Barrera et al. (2013), fat in chicken breast meat contains 33.5% of saturated, 30.5% unsaturated and 32% polyunsaturated fatty acids, which encouraging human consumption when these are compared with the low levels of PUFA and high levels of SFA in red meats. The reported health benefits of PUFA on human health have created a trend toward replacement of saturated fats with unsaturated fats in poultry products through dietary manipulation (Ahmed et al., 2015). The manipulating the ratio of these FAs could be an effective way to improve the consumer's health and to make chicken meat even more attractive to consumers (Taulescu et al., 2010).

The fatty acids profile of poultry meat is influenced by internal (age, gender, and genotype) and external (temperature, feeding) factors (Starčević et al., 2014), therefore the dietary alterations can be used to modify the proportion of PUFA in chicken meat (Rymer and Givens, 2005). Fatty acids are involved in technological aspects of meat quality. The components of technological meat quality influenced by FA are fat tissue firmness (hardness), shelf life (lipid and pigment oxidation) and flavour. Owing to very different melting points of fatty acids, variation in their composition has also an important effect on firmness or softness of the fat in meat, especially the subcutaneous, intermuscular, and intramuscular fat (Wood et al., 2003; Nieto and Ros, 2012). On the other hand, from a sensory point of view, FAs are major precursors of meat flavour compounds which resulted from oxidation of FAs during long-term storage or meat cooking process. Unsaturated FA undergo autoxidation much more readily than those which are saturated. High levels of long

chain PUFA or n-3 FA would thus significantly decrease the oxidative stability and consequently negatively affect meat flavour and colour (Zhou et al., 2012) that may then lead to lower consumer acceptability (Bou et al., 2004).

Nevertheless, FA composition of chicken meat is influenced by the diet provided to chickens. Therefore, aim of the present study was to investigate how the diets for broiler chickens supplemented with propolis separately and propolis together with probiotics would affect the FA profile in breast and thigh muscles.

Material and methods

Animals and diet

The experiment was carried out in test poultry station of Slovak University of Agriculture in Nitra. A total of 180 one day-old broiler chicks of mixed sex (Ross 308) were randomly divided into 3 groups, namely, control (C) and experimental (E1, E2).

Table 1. Composition of basal diet

Tabuľka 1. Zloženie kŕmnych zmesí

Ingredients (%)	Starter phase (1 to 21 d)	Grower phase (22 to 42 d)
Wheat	35	35
Maize	35	40
Soybean meal (48% CP)	21.3	18.7
Fish meal (71% CP)	3.8	2
Dried blood	1.25	1.25
Ground limestone	1	1.05
Monocalcium phosphate	1	0.7
Fodder salt	0.1	0.15
Sodium bicarbonate	0.15	0.2
Lysine	0.05	0.07
Methionine	0.15	0.22
Palm kernel oil Bergafat	0.7	0.16
Premix Euromix BR 0.5%[1]	0.5	0.5

CP – crude protein; [1]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.

CP – dusíkaté látky; [1]obsah účinných látok v 1 kg premixu: vitamín A 2 500 000 m.j.; vitamín E 20 000 mg; vitamin D3 800 000 m.j.; niacín 12 000 mg; kyselina D-pantoténová 3 000 mg; riboflavín 1 800 mg; pyridoxín 1 200 mg; tiamín 600 mg; menadión 800 mg; kyselina askorbová 20 000 mg; kyselina listová 400 mg; biotín 40 mg; kobalamín 8.0 mg; cholín 100 000 mg; betaín 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.

Each group consisted of 3 replicated pens with 20 broiler chickens per pen. The experiment employed a randomized design, and dietary treatments were as follows: 1. basal diet as control (C group), 2. basal diet plus 400 mg propolis extract per 1 kg of feed mixture (E1 group), 3. basal diet plus 400 mg propolis extract per 1 kg of feed mixture and 3.3 g probiotic preparation added to drinking water (E2 group). Besides, the groups were kept under the same conditions.

Table 2. Nutrient content of basal diet

Tabuľka 2. Obsah živín v kŕmnych zmesiach

Nutrient content ($g*kg^{-1}$)	Starter phase (1 to 21 d)	Grower phase (22 to 42 d)
Crude protein (CP)	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
Linoleic acid	13.51	14.19
ME_N ($MJ*kg^{-1}$)	12.02	12.03

CP – crude protein; ME_N – nitrogen-corrected metabolizable energy; MJ – megajoule.

CP – dusíkaté látky; ME_N – metabolizovateľná energia upravená na dusíkovú rovnováhu; MJ – megajoul.

The broilers were reared on breed litter (wood shavings) up to 42 days of age. Room temperature was adjusted at 33 °C in the first week and gradually decreased by 2 °C, and finally fixed at 19 °C thereafter. Broilers had *ad libitum* access to feed and water, and lighting was provided continuously.

Tables 1 and 2 lists the ingredients and nutrient content of the basal diet, formulated to provide the nutrient requirements of broilers according to the recommended reference levels. The diets were offered in two phases: starter from 1 to 21 days (HYD-01, powder form) and grower from 22 to 42 days (HYD-02, powder form). The feed mixtures both starter and grower were produced without any antibiotics and coccidiostats.

In the experiment, the probiotic preparation based on *Lactobacillus fermentum* ($1*10^9$ CFU in 1 g of bearing medium) was used.

Propolis had origin in the Slovak Republic. The extract was prepared from minced propolis in the conditions of the 80% ethanol in the 500 cm^3 flasks, according to Krell (1996).

Slaughter and measurements

At the end of the 42-d feeding period, broilers were weighed and slaughtered at the slaughterhouse of Slovak University of Agriculture in Nitra. After evisceration, the carcasses were kept at approximately 18 °C for 1 h *post mortem* and thereafter

longitudinally divided into two parts. After that, the half-carcasses and giblets were weighed and stored at 4 °C until 24 h *post mortem*. The right half-carcasses were used in order to determinate the FA composition, whereas the left half-carcasses were assigned to different analysis. The breast and thigh muscles were separated from each half-carcass for the determination the FA composition. The FA compositions of breast and thigh meats were determined by a direct method for fatty acid methyl ester (FAME) synthesis. The FA composition of the FAME was determined using a Gas Chromatograph (Agilent, 7890A series, USA) equipped with a flame ionization detector and a chiral capillary column (J&W Scientific, USA).

Statistical analysis

All statistical analyses were computed using the ANOVA procedures of SAS software (version 9.3, SAS Institute Inc., USA, 2008). Mean values and standard deviation (SD) are reported in tables. Statistical significance was calculated using t-test. Differences between the treatments were considered significant at $P \leq 0.05$.

Results and discussion

As shown in Table 3, 6 out of 15 fatty acids that were detected in broiler breast meat differed ($P \leq 0.05$) in proportion among the treatments. Breast meat from the control treatment had a higher ($P \leq 0.05$) concentration of stearic acid than did breast meat from the propolis and propolis + probiotic-supplemented groups (E2).

The highest ($P > 0.05$) concentration of palmitoleic acid was found in propolis + probiotic-supplemented group (E2). In addition, breast meat from this treatment had the lowest ($P \leq 0.05$) concentration of stearic, linoleic, eicosadienoic, and arachidonic acids. The highest ($P \leq 0.05$) linoleic and arachidonic content was found in breast meat from chickens fed propolis (E1). The higher linoleic and arachidonic acid contents of breast meat can be an attractive nutritional quality trait for health-conscious consumers. The propolis + probiotic-supplemented group (E2) also showed the lowest ($P \leq 0.05$) proportion of PUFA. On the contrary, this treatment showed the highest ($P \leq 0.05$) proportion of MUFA. No significant difference ($P > 0.05$) was found in n-3:n-6 PUFA and n-6:n-3 PUFA ratio.

As shown in Table 4, only 4 of 15 fatty acids that were detected in broiler thigh meat differed ($P \leq 0.05$) in proportion among the treatments. The addition of both supplements (propolis separately and propolis together with probiotics) had a significant effect on two saturated FA, namely the myristic and palmitic acids, due to the highest ($P \leq 0.05$) concentration in control.

The supplements had not significant ($P > 0.05$) effect on stearic acid content, which was, however, lower in both supplemented group. The lowest ($P \leq 0.05$) SFA proportion was found in propolis-supplemented group (E1). Feeding with propolis+probiotics (E2) had a tendency to decrease SFA ($P \leq 0.05$), as well as PUFA ($P > 0.05$) proportion. In addition, thigh muscle from this treatment (E2) had relatively high ($P > 0.05$) MUFA level when compared with the other treatments. The significant changes ($P \leq 0.05$) were also found in the n-3:n-6 PUFA and n-6:n-3 PUFA ratios.

Table 3. The fatty acids profile (g*100 g^{-1}) of chicken breast muscle (mean±SD)

Tabuľka 3. Profil mastných kyselín (g*100 g^{-1}) v prsnej svalovine kurčiat (mean±SD)

Fatty acid	C	E1	E2
Myristic (C14:0)	0.7±0.022	0.68±0.011	0.67±0.039
Pentadecanoic (C15:0)	0.07±0.058	0.09±0.047	$0.11±3.64*10^{-3}$
Palmitic (C16:0)	26.72±0.512	26.6±0.384	26.84±0.55
Heptadecanoic (C17:0)	$0.11±0.052^{ab}$	$0.12±6.933*10^{-3\ a}$	$0.11±6.911*10^{-3\ b}$
Stearic (C18:0)	$6.85±0.247^{a}$	$6.6±0.341^{ab}$	$6.35±0.256^{b}$
Myristoleic (C14:1)	0.2±0.012	0.19±0.014	0.21±0.03
Palmitoleic (C16:1)	6.51±0.274	6.45±0.397	7.01±0.658
Oleic (C18:1 cis)	40.35±1	40.18±0.681	40.99±1.362
Linoleic (C18:2 cis)	$11.57±0.695^{ab}$	$12.02±0.518^{a}$	$11.34±0.548^{b}$
Linolenic (C18:3 n-6)	0.11±0.044	0.13±0.021	0.12±0.01
Linolenic (C18:3 n-3)	0.74±0.027	0.77±0.034	0.74±0.034
Eicosenoic (C20:1)	0.49±0.028	0.47±0.016	0.46±0.025
Eicosadienoic (C20:2)	$0.27±0.031^{a}$	$0.27±0.025^{a}$	$0.22±0.019^{b}$
Eicosatrienoic (C20:3)	$0.22±0.029^{a}$	$0.22±0.029^{a}$	$0.18±0.017^{b}$
Arachidonic (C20:4)	$0.54±0.104^{a}$	$0.56±0.07^{a}$	$0.37±0.073^{b}$
Σ SFA	34.45±0.693	34.1±0.378	34.07±0.418
Σ MUFA	$48.12±0.962^{ab}$	$47.84±0.563^{a}$	$49.09±0.887^{b}$
Σ PUFA	$13.64±0.848^{ab}$	$14.15±0.549^{a}$	$13.11±0.696^{b}$
n-3 PUFA:n-6 PUFA	$0.07±2.115*10^{-3}$	$0.07±1.847*10^{-3}$	$0.07±9.607*10^{-3}$
n-6 PUFA:n-3 PUFA	13.77±0.409	14.01±0.362	13.92±0.183

C – control group; E1, E2 – experimental groups; SD – standard deviation; a, b – means with different superscripts within a row differ significantly (P ≤ 0.05).

C – kontrolná skupina; E1, E2 – pokusné skupiny; SD – smerodajná odchýlka; priemerné hodnoty označené rozdielnymi písmenami (a, b) v riadku sú preukazné na hladine významnosti P ≤ 0.05.

Milićević et al. (2014) investigated the impact of chicken meat consumption on cardiovascular risk in the general population and reported main FA in chicken meat as follows: oleic (C18:1 cis), linoleic (C18:2 cis), palmitic (C16:0), stearic (C18:0), and palmitoleic (C16:1). Oleic acid was observed at highest level (42.85%) in both breast and thigh muscles. In breast muscles, the major SFA was palmitic acid and ranged from 21.35% to 28.53%; and the major PUFA was linoleic acid and varied from 10.26% to 24.85%. The fatty acids profile of thigh muscle showed a slightly higher fraction of PUFA (C18:2 cis) in comparison to SFA (16:0). In accordance with these data, it was determined in the present study the similar FA profile of chicken meat. Oleic acid was detected at level of 40.18 – 40.99% and 41.12 – 41.85% in breast muscle and thigh muscles, respectively, which is slightly lower value as compared to study of Milićević et al. (2014).

Table 4. The fatty acids profile (g*100 g^{-1}) of chicken thigh muscle (mean±SD)

Tabuľka 4. Profil mastných kyselín (g*100 g^{-1}) v stehennej svalovine kurčiat (mean±SD)

Fatty acid	C	E1	E2
Myristic (C14:0)	0.69±0.025^{a}	0.66±0.011^{b}	0.65±0.047^{ab}
Pentadecanoic (C15:0)	0.1±5.55*10^{-3}	0.1±4.29*10^{-3}	0.1±4.103*10^{-3}
Palmitic (C16:0)	27.05±0.497^{a}	26.42±0.376^{b}	26.83±0.916^{ab}
Heptadecanoic (C17:0)	0.1±4.952*10^{-3}	0.11±6.76*10^{-3}	0.1±7.166*10^{-3}
Stearic (C18:0)	5.94±0.25	5.63±0.264	5.85±0.208
Myristoleic (C14:1)	0.22±0.015	0.22±0.015	0.22±0.037
Palmitoleic (C16:1)	7.52±0.426	7.47±0.44	7.65±0.866
Oleic (C18:1 cis)	41.12±0.58	41.47±0.827	41.85±1.826
Linoleic (C18:2 cis)	11.41±0.347^{ab}	11.82±0.436^{a}	11.05±0.686^{b}
Linolenic (C18:3 n-6)	0.13±0.012	0.13±0.013	0.12±0.014
Linolenic (C18:3 n-3)	0.76±0.015	0.79±0.026	0.77±0.037
Eicosenoic (C20:1)	0.42±0.015	0.42±0.015	0.43±0.028
Eicosadienoic (C20:2)	0.15±0.011^{a}	0.16±5.023*10$^{-3\ b}$	0.16±0.018^{ab}
Eicosatrienoic (C20:3)	0.1±9.594*10^{-3}	0.11±6.525*10^{-3}	0.11±0.015
Arachidonic (C20:4)	0.2±0.03	0.25±0.029	0.24±0.048
Σ SFA	33.89±0.582^{a}	32.92±0.389^{b}	33.53±0.835^{ab}
Σ MUFA	49.57±0.316	49.87±0.572	50.46±1.1
Σ PUFA	12.87±0.411	13.38±0.469	12.56±0.809
n-3 PUFA:n-6 PUFA	0.07±1.17*10$^{-3\ a}$	0.07±1.692*10$^{-3\ a}$	0.08±1.226*10$^{-3\ b}$
n-6 PUFA:n-3 PUFA	13.7±0.217^{a}	13.57±0.318^{a}	13.22±0.217^{b}

C – control group; E1, E2 – experimental groups; SD – standard deviation; a, b – means with different superscripts within a row differ significantly (P ≤ 0.05).

C – kontrolná skupina; E1, E2 – pokusné skupiny; SD – smerodajná odchýlka; priemerné hodnoty označené rozdielnymi písmenami (a, b) v riadku sú preukazné na hladine významnosti P ≤ 0.05.

In the present study, there was found much higher palmitic acid content in breast muscle that varied from 26.6 – 26.84%, as well as in thigh muscle (26.42 – 27.05%). The major PUFA was linoleic acid in breast and thigh muscles (11.34 – 12.02% and 11.05 – 11.82%, respectively). Although the n-3:n-6 FA ratio (0.07 – 0.08%) was similar in breast and thigh muscles for dietary groups, n-6:n-3 ratio of meat (13.77 – 14.01% in breast; 13.22 – 13.7% in thigh) were different between the samples analyzed. The high n-6:n-3 ratio may be due to the predominance of wheat, maize, and soy in feed mixtures given to broilers, which is relatively high in n-6 fatty acids when compared with n-3 fatty acids.

There has been much debate over the practical utility of the dietary ratio of n-6 to n-3 PUFA in optimizing the benefits of n-3 PUFA on cardiovascular health. According to Simopolous (2002), excessive amounts of n-6 PUFA and a very high n-6 PUFA:n-3

PUFA ratio promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of n-3 PUFA (a low n-6:n-3 ratio) exert suppressive effects. The study indicated that the optimal ratio may vary with the disease under consideration.

Similar arguments were reported by Deckelbaum (2010), who noticed that for both n-3 and n-6 FA, adequate intake amounts for each are likely to be of higher utility than consideration of the n-6:n-3 ratio. The ratio can disguise extremely low or high intakes of n-6 and/or n-3 FA.15 Moreover, the specific n-6 and n-3 FA that are represented in calculating an n-6:n-3 ratio are not always clearly defined.

Results of the present study indicated that supplementation of chicken diet with the feed additives had slight effect on fatty acids profile of breast and thigh muscles. More obvious results were achieved in breast muscle, in total MUFA and PUFA content in particular. It is noteworthy that the highest ($P \leq 0.05$) linoleic and arachidonic acid proportions in breast meat of chickens that were fed with propolis extract (E1) may be due to high essential fatty acids content in the propolis (Zhang et al., 2005; Wang et al., 2014; Li et al., 2016).

The effect of propolis in diet of broilers on the total PUFA content was shown to be favourable ($P \leq 0.05$) when the propolis was administered singly (E1), but rather adverse ($P > 0.05$) when administered together with probiotics (E2). Regarding this fact it can be assumed that after inclusion of propolis in diet of broilers it would be possible to produce chicken meat with a high content of n-3 PUFA as a functional food.

However, the impact cannot be so obvious like after dietary supplementation of additives that are typically high in n-3 PUFA content such is linseed oil. The effect of dietary linseed oil on content of fatty acids in chicken meat was investigated in many studies (López-Ferrer et al., 2001; Zelenka et al., 2008; Taulescu et al., 2010; Mandal et al., 2014; Mašek et al., 2014;), where linseed oil supplementation significantly ($P \leq 0.05$) increased linolenic acid content and total PUFA n-3 content, and decreased arachidonic acid and total PUFA n-6, and n-6 PUFA:n-3 PUFA ratio.

The effect of propolis extract on fatty acids profile in crossbred bulls was objective of the study of Valero et al. (2011). The inclusion of propolis extract in diet has only little impact ($P > 0.05$) on FA composition of the *Longissimus* muscle of crossbred bulls. In the study, PUFA, MUFA, SFA, n-6, n-3 FA or the ratio of PUFA:SFA and n-6:n-3 PUFA were not changed. The effect was observed only for two FA of the 20 analyzed, namely an increase in linoleic acid and decrease in docosahexaenoic acid. Another study (Valero et al., 2014) also showed a minimal effect on *Longissimus* muscle FA composition when propolis was included in the diet of crossbred bulls.

Similarly, there was the effect of other honey bee products on FA composition investigated. Seven et al. (2014) reported improved unsaturated FA ratios after royal jelly supplementation in quails. In the study of Tatlı Seven et al. (2016), bee pollen supplementation in Japanese quails increased PUFA levels in tissues, especially n-6 PUFA.

In another study (Schilling et al., 2010), effects of feeding various levels of distillers dried grains with soluble (DDGS) were evaluated. Thigh meat was evaluated for FA composition. Feeding DDGS had a tendency to increase linoleic and linolenic acid,

and decrease the proportions of palmitic and oleic acid when compared with the control.

Also, Min et al. (2012) evaluated the effects of DDGS on FA profiles of breast and thigh muscles. Dietary addition of DDGS led to significant increase ($P \leq 0.05$) in the ratio of PUFA:SFA ratio in both breast and thigh muscles.

Zduńczyk et al. (2011) reported that diet containing the addition of selenium and vitamin E in broilers had no significant effect ($P > 0.05$) on FA profile of breast muscle.

In the study of Straková et al. (2010), lupin meal in chicken diet resulted in decrease of SFA in breast and thigh muscles. Slight decrease was also observed in n-6 PUFA. On the other hand, diets containing lupin meal showed an increase in MUFA and n-3 PUFA.

Gallardo et al. (2012) found an increase in oleic acid ($P \leq 0.05$), and a decrease in linoleic acid ($P \leq 0.05$), together with a slight increase in α-linolenic acid ($P \leq 0.05$) in broiler chickens fed canola oil, which is high in MUFA.

Ahmed et al. (2015) detected lower SFA, higher MUFA and n-3 FA in breast and thigh muscles after dietary supplementation with pomegranate by-product (PB) in chickens. They also found lower ($P \leq 0.05$) n-6:n-3 ratio in the 1.0% and 2.0% PB-supplemented group.

Conclusions

The results of this study showed that the supplementation of chicken diet with propolis separately and propolis together with probiotics affect the fatty acids profile of breast and thigh muscles. The most changes in FA profiles of breast and thigh muscles were observed after propolis supplementation (administered separately), which is probably related to a high proportion of FAs (oleic, palmitic, linoleic, and stearic) identified as a part of propolis chemical composition. The findings offered important evidence for further research on the addition of propolis into chicken diet so as to improve the meat quality.

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References

Ahmed, S. T., Islam, M. M., Bostami, A. B. M. R., Mun, H., Kim, Y., Yang, C. (2015) Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum* L.) by-products. Food Chemistry, 188, 481-488. DOI: http://dx.doi.org/10.1016/j.foodchem.2015.04.140

Bou, R., Guardiola, F., Tres, A., Barroeta, A. C., Codony, R. (2004) Effect of dietary fish oil, α-tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat. Poultry Science, 83 (2), 282-292. DOI: http://dx.doi.org/10.1093/ps/83.2.282

Cortinas, L., Villaverede, C., Galobart, J., Baucells, M. D., Codony, R., Barroeta, A. C. (2004) Fatty Acid Content in Chicken Thigh and Breast as Affected by Dietary Polyunsaturation Level. Poultry Science, 83 (7), 1155-1164. DOI: http://dx.doi.org/10.1093/ps/83.7.1155

Dalziel, C. J., Kliem, K. E., Givens, D. I. (2015) Fat and fatty acid composition of cooked meat from UK retail chickens labelled as from organic and non-organic production systems. Food Chemistry, 179, 103-108. DOI: http://dx.doi.org/10.1016/j.foodchem.2015.01.118

Deckelbaum, R. J. (2010) n-6 and n-3 Fatty Acids and Atherosclerosis Ratios or Amounts? Arteriosclerosis, Thrombosis, and Vascular Biology, 30 (12), 2325-2326. DOI: http://dx.doi.org/10.1161/atvbaha.110.214353

Gallardo, M. A., Pérez, D. D., Leighton, F. M. (2012) Modification of fatty acid composition in broiler chickens fed canola oil. Biological Research, 45 (2), 149-161. DOI: http://dx.doi.org/10.4067/s0716-97602012000200007

Gibbs, R. A., Rymer, C., Givens, D. I. (2013) Fatty acid composition of cooked chicken meat and chicken meat products as influenced by price range at retail. Food Chemistry (2-3), 138, 1749-1756. DOI: http://dx.doi.org/10.1016/j.foodchem.2012.11.002

Krell, R. (1996) Value-added products from bee keeping. Rome : FAO.

Li, H., Wu, F., Tan, J., Wang, K., Zhang, C., Zheng, H., Hu, F. (2016) Caffeic acid phenethyl ester exhibiting distinctive binding interaction with human serum albumin implies the pharmacokinetic basis of propolis bioactive components. Journal of Pharmaceutical and Biomedical Analysis, 122, 21-28. DOI: http://dx.doi.org/10.1016/j.jpba.2016.01.040

López-Ferrer, S., Baucells, M. D., Barroeta, A. C, Galobart, J, Grashorn, M. A. (2001) n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: linseed oil. Poultry Science, 80 (6), 753-761. DOI: http://dx.doi.org/10.1093/ps/80.6.753

Mandal, G. P., Ghosh, T. K, Patra, A. K. (2014) Effect of Different Dietary n-6 to n-3 Fatty Acid Ratios on the Performance and Fatty Acid Composition in Muscles of Broiler Chickens. Asian-Australasian Journal of Animal Sciences, 27 (11), 1608-1614. DOI: http://dx.doi.org/10.5713/ajas.2014.14013

Mapiye, C., Chimonyo, M., Dzama, K., Hugo, A., Strydom, P. E., Muchenje, V. (2011) Fatty acid composition of beef from Nguni steers supplemented with *Acacia karroo* leaf-meal. Journal of Food Composition and Analysis, 24 (4-5), 523-528. DOI: http://dx.doi.org/10.1016/j.jfca.2011.01.018

Mašek, T., Starčević, K., Filipović, N., Stojević, Z., Brozić, D., Gottstein, Z., Severin, K. (2014) Tissue fatty acid composition and estimated Δ desaturase activity after castration in chicken broilers fed with linseed or sunflower oil. Journal of Animal Physiology and Animal Nutrition, 98 (2), 384.392. DOI: http://dx.doi.org/10.1111/jpn.12114

Milićević, D., Vranić, D., Mašić, Z., Parunović, N., Trbović, D., Nedeljković-Trailović, J., Petrović, Z. (2014) The role of total fats, saturated/unsaturated fatty acids and cholesterol content in chicken meat as cardiovascular risk factors. Lipids in Health and Diseas, 13 (1), 42. DOI: http://dx.doi.org/10.1186/1476-511x-13-42

Min, Y. N., Li, L., Waldroup, P. W., Niu, Z. Y., Wang, Z. P., Gao, Y. P., Liu F. Z. (2012) Effects of dietary distillers dried grains with solubles concentrations on meat quality and antioxidant status and capacity of broiler chickens. The Journal of Applied Poultry Research, 21 (3), 603-611. DOI: http://dx.doi.org/10.3382/japr.2011-00503

Morales-Barrera, J. E., Gonzalez-Alcorta, M. J., Castillo-Dominguez, R. M., Prado-Rebolledo, O. F., Hernandez-Velasco, X., Menconi, A., Tellez, G., Hargis, B. M., Carrillo-Dominguez, S. (2013) Fatty Acid Deposition on Broiler Meat in Chickens Supplemented with Tuna Oil. Food and Nutrition Sciences, 4 (9), 16-20. DOI: http://dx.doi.org/10.4236/fns.2013.49a1003

Nieto, G., Ros, G. (2012) Modification of Fatty Acid Composition in Meat Through Diet: Effect on Lipid Peroxidation and Relationship to Nutritional Quality – A Review. In: A. Catala, ed. (2012) Lipid Peroxidation. Rijeka : InTech. 239-258. ISBN 978-953-51-0716-3.

Nkukwana, T. T., Muchenje, V., Masika, P. J., Hoffman, L. C., Dzama, K., Descalzo, A. M. (2014) Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with *Moringa oleifera* leaf meal over a period of refrigeration. Food Chemistry, 142, 255-261. DOI: http://dx.doi.org/10.1016/j.foodchem.2013.07.059

Parunović, N., Petrović, M., Matekalo-Sverak, V., Trbović, D., Mijatović, M., Radović, Č. (2012) Fatty acid profile and cholesterol content of *m. longissimus* of free-range and conventionally reared. South African Journal of Animal Science, 42 (2), 101-113. DOI: http://dx.doi.org/10.4314/sajas.v42i2.2

Rymer, C., Givens, D. I. (2005) n-3 fatty acid enrichment of edible tissues of poultry: A review. Lipids, 40 (2), 121-140. DOI: http://dx.doi.org/10.1007/s11745-005-1366-4

SAS (2008). 9.3 Enhanced Logging Facilities. [Computer software].Cary, NC : SAS Institute Inc.

Schilling , M. W., Battula, V., Loar II, R. E., Jackson, V., Kin, S., Corzo, A. (2010) Dietary inclusion level effects of distillers dried grains with soluble on broiler meat quality. Poultry Science, 89 (4), 752-760. DOI: http://dx.doi.org/10.3382/ps.2009-00385

Seven, İ., Şimşek, Ü, G., Gökçe, Z., Tatli Seven, P., Arslan, A., Yilmaz, Ö. (2014) The effects of royal jelly on performance and fatty acid profiles of different tissues in quail (*Coturnix coturnix japonica*) reared under high stocking density. Turkish Journal of Veterinary and Animal Sciences, 38, 271-277. DOI: http://dx.doi.org/10.3906/vet-1303-62

Simopoulos, A. P. (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy, 56 (8), 365-379. DOI: http://dx.doi.org/10.1016/s0753-3322(02)00253-6

Soriano-Santos, J. 2010. Chemical Composition and Nutritional Content of Raw Poultry Meat. In: I. Guerrero-Legarreta, ed. (2010) Handbook of Poultry Science and Technology, Volume 1, Primary Processing. Hoboken : John Wiley & Sons. 467-491. ISBN 978-0-470-18552-0.

Starčević, K., Mašek, T., Brozić, D., Filipović, N., Stojević, Z. (2014) Growth performance, serum lipids and fatty acid profile of different tissues in chicken broilers fed a diet supplemented with linseed oil during a prolonged fattening period. Veterinarski Arhiv, 84 (1), 75-84.

Straková, E., Suchý, P., Herzig, I., Hudečková P., Ivanko Š. (2010) Variation in fatty acids in chicken meat as a result of a lupin-containing diet. Czech Journal of Animal Science, 55 (2), 75-82.

Tatlı Seven, P., Arslan, A. S., Seven, İ., Gökçe, Z. (2016) The effects of dietary bee pollen on lipid peroxidation and fatty acids composition of Japanese quails (*Coturnix coturnix japonica*) meat under different stocking densities. Journal of Applied Animal Research, 44 (1), 487-491. DOI: http://dx.doi.org/10.1080/09712119.2015.1091339

Taulescu, C., Mihaiu, M., Bele, C., Matea, C., Dan, S. D., Mihaiu, R., Lapusan, A., Ciupa, A. (2010) Manipulating the Fatty Acid Composition of Poultry Meat for Improving Consumer's Health. Bulletin UASVM, Veterinary Medicine, 67, 220-225.

Valero, M. V., Zawadzki, F., Françozo, M. C., de Souza Farias, M., Rotta, P. P., Prado, I. N., Visantainer, J. V., Zeoula, L. M. (2011) Sodium monensin or propolis extract in the diet of crossbred (½ Red Angus vs. ½ Nellore) bulls finished in feedlot: chemical composition and fatty acid profile of the *Longissimus* muscle. Semina: Ciências Agrárias, 32 (4), 1617-1626. DOI: http://dx.doi.org/10.5433/1679-0359.2011v32n4p1617

Valero, M. V., Torrecilhas, J. A., Zawadzki, F., Bonafé, E. G., Madrona, G. S., do Prado, R. M., Passetti, R. A. C., Rivaroli, D. C., Visentainer, J. V., do Prado, I. N. (2014) Propolis or cashew and castor oils effects on composition of Longissimus muscle of crossbred bulls finished in feedlot. Chilean Journal of Agricultural Research, 74 (4), 445-451. DOI: http://dx.doi.org/10.4067/s0718-58392014000400011

Wang, Z., Sun, R., Wang, Y., Li, N., Lei, L., Yang, X., Yu, A., Qiu, F., Zhang, H. (2014) Determination of phenolic acids and flavonoids in raw propolis by silica-supported ionic liquid-based matrix solid phase dispersion extraction high performance liquid chromatography-diode array detection. Journal of Chromatography B, 969, 205-212. DOI: http://dx.doi.org/10.1016/j.jchromb.2014.08.022

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R., Enser, M. (2003) Effects of fatty acids on meat quality: a review. Meat Science, 66 (1), 21-32. DOI: http://dx.doi.org/10.1016/s0309-1740(03)00022-6

Zduńczyk, Z., Gruzauskas, R., Semaskaite, A., Juskiewicz, J., Raceviciute-Stupeliene, A., Wroblewska, M. (2011) Fatty acid profile of breast muscle of broiler chickens fed diets with different levels of selenium and vitamin E. Archiv für Geflügelkunde, 75 (4), 264-267.

Zelenka, J., Schneiderova, D., Mrkvicova, E., Dolezal, P. (2008) The effect of dietary linseed oils with different fatty acid pattern on the content of fatty acids in chicken meat. Veterinarni Medicina, 53 (2), 77-85.

Zhang, S. Q., Xi, J., Wang, C. Z. (2005) High hydrostatic pressure extraction of flavonoids from propolis. Journal of Chemical Technology and Biotechnology, 80 (1), 50-54. DOI: http://dx.doi.org/10.1002/jctb.1153

Zhou, L. J., Wu, H., Li, J. T., Wang, Z. Y., Zhang, L. Y. (2012) Determination of fatty acids in broiler breast meat by near-infrared reflectance spectroscopy. Meat Science, 90 (3), 658-664. DOI: http://dx.doi.org/10.1016/j.meatsci.2011.10.010