Original scientific paper
DOI: /10.5513/JCEA01/19.4.2050
Journal of Central European Agriculture, 2018, 19(4), p.931-942

Cholesterol content and fatty acid profile of broiler meat as affected by diet with extruded flaxseed

Uticaj ishrane sa dodatkom ekstrudiranog semena lana na sadržaj holesterola i sastav masnih kiselina mesa brojlera

Dušan ŽIVKOVIĆ[1], Slobodan LILIĆ[2], Slaviša STAJIĆ[1]*, Danijela VRANIĆ[2], Dejana TRBOVIĆ[2] and Nikola Stanišić[3]

[1]University of Belgrade, Faculty of Agriculture, Department of Animal Source Food Technology, Nemanjina 6, 11080, Belgrade, Serbia, *correspondence: stajic@agrif.bg.ac.rs

[2]Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia

[3]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade, Serbia

Abstract

To improve the fatty acid (FA) composition of meat, supplements rich in n-3 polyunsaturated fatty acids (PUFA) can be added to animal diet. The aim of this research was to examine cholesterol content and FA profile of white (breast) and dark meat (leg-thigh) by feeding two groups [both with males (M) and females (F)] of 1000 broilers of the Ross-308 hybrid line by standard feed (control group – CONM and CONF) and with the addition of 6% of extruded flaxseed mixture (experimental group – EXPM and EXPF). The addition of extruded flaxseed to the diet of broilers did not affect ($P>0.05$) the content of cholesterol in meat, which was between 46.13 and 52.94 mg·100 g^{-1} in white meat and 51.31–55.47 in dark meat. The content of α-linolenic acid increased significantly ($P<0.01$) in both white [0.72(CONF)−1.62(CONM) to 2.2(EXPF)–3.03(EXPM) % total FA] and dark meat [1.75(CONF)–1.98(CONM) to 4.47(EXPM)–4.66(EXMF) % total FA], as did the content of n-3 FA and PUFA, while the n-6/n-3 ratio decreased, reaching values below 6. The effect was more pronounced in the meat of females and in dark meat. By adding extruded flaxseed to broilers feed the nutritional characteristics of meat can be improved.

Keywords: α-linolenic acid, broiler meat, cholesterol, extruded flaxseed, n-3 fatty acids

Sažetak

Uvođenjem sastojaka bogatih n-3 polinezasićenim masnim kiselinama (n-3 PUFA) u ishranu životinja može se poboljšati sastav masnih kiselina mesa. Cilj ovog ogleda je

da se ispita uticaj ishrane obogaćene ekstrudiranim semenom lana na sadržaj holesterola i sastav masnih kiselina belog mesa (grudi) i tamnog mesa (batak sa karabatkom) pilića. Korišćene su dve gupe sa 1000 pilića (obe sa mužjacima i ženkama) Ros-308 hibridne linije koji su hranjeni standardnom ishranom (kontrolna grupa: mužjaci – CONM i ženke – CONF) i hranom sa 6% komercijalne smeše koja sadrži ekstrudirano seme lana (eksperimentalna grupa: mužjaci – EXPM i ženke − EXPF). Dodatak ekstrudiranog semena lana u ishranu pilića nije značajno (P>0,05) uticao na sadržaj holesterola u mesu, koji je bio u intervalu 46,12–52,94 mg·100 g^{-1} belog mesa i 51,31–55,47 mg·100 g^{-1} tamnog mesa. Sadržaj α-linoleinske kiseline značajno je bio veći u eksperimentalnoj grupi i u belom mesu [0,72(CONF)−1,62(CONM) prema 2,2(EXPF)–3,03(EXPM) % ukupnih masnih kiselina] i u tamnom mesu [1,75(CONF)–1,98(CONM) prema 4,47(EXPM)−4,66(EXMF) % ukupnih masnih kiselina], kao i sadržaj n-3 masnih kiselina i sadržaj PUFA, dok je odnos n-6/n-3 opao na vrednosti manje od 6. Uticaj je bio izraženiji u tamnom mesu i u mesu ženki. Dodatkom ekstrudiranog semena lana u ishranu pilića mogu se značajno poboljšati nutritivna svojstva mesa.

Keywords: α-linoleinska kiselina, ekstrudirano sema lana, holesterol, n-3 masne kiseline, pileće meso

Introduction

During the past decade more attention has been paid to improving nutrition by increasing the beneficial aspects of food and reducing or eliminating the negative ones. The favorable health effects of n-3 polyunsaturated fatty acids (n-3 PUFA) taken in through food (plants, fish and seafood) are well-known, as is the importance of the n-6/n-3 fatty acids ratio (Campioli et al., 2012). However, meat lipids do not have such good characteristics. They had a low content of n-3 PUFA, as well as an unfavorable ratio of n-6/n-3 FA (Hathwar et al., 2012). Poultry meat contains less fat than most cuts of beef and pork, but is relatively poor in n-3 PUFA when animals are fed with standard diets (Bou et al., 2009). Regarding meat consumption and production in the last 5–6 decades, poultry meat has been gaining importance in human nutrition and nowadays its production accounts for more than 1/3 of global meat production. Chickens are the most common source of poultry meat (FAO, 2013).

Nutrition is the easiest way to influence the composition and quality of muscle and fatty tissues in monogastric animals (pigs, poultry and fish) since their organism absorbs fatty acids in their intact form (Živković et al., 2013). Flaxseed oil added to poultry diet up to 4% had a positive effect on n-3 PUFA meat content without altering its sensory characteristics (López-Ferrer et al., 2001a; Panda et al., 2015).

Although flaxseed is an excellent source of oil, protein, fibre, lecithin, phenols vitamins and minerals, until recently, its use was limited by anti-nutritional factors such as: cyanogenic glycosides, phytic acid, linatin dipeptide (Betti et al., 2009a; Anjum et al., 2013). These anti-nutritional factors can be reduced by the extrusion process, which makes extruded flaxseed adequate for use in animal diet. Despite that, however, some research studies indicate that diet with 5% or more of extruded

flaxseed reduces body weight gain and feed intake and increases the feed conversion ratio (Anjum et al., 2013). On the other hand, Shafey et al. (2014) state that a diet with up to 8% of flaxseed meal did not affect body weight gain, feed intake and the feed conversion ratio. Furthermore, some research studies suggest that poultry diet with 5% or more of extruded flaxseed could have a negative effect on oxidative stability and some sensory characteristics of broiler meat such as flavor and aroma (Anjum et al., 2013).

The goal of this experiment was to examine the effect of the addition of 3% of extruded flaxseed to broiler feed on some nutritional characteristics – cholesterol content and fatty acids profile of breast and leg-thigh meat as the most valuable parts in nutritional and economic terms.

Materials and methods

Dietary treatments and broiler husbandry

This research is a follow-up of previous research by Živković et al. (2017), therefore the dietary treatments (starter and finisher), broiler husbandry and experimental design were the same as described in that research. Briefly, one thousand unsexed one-day broilers of the Ross–308 hybrid line had ad libitum access to water and to the diets (starter to 28 days, followed by 28–45 day finisher). On day 28, two equal groups were randomly formed in three replicates each, having an approximately equal sex ratio: control group (CON), fed by standard feed, and experimental group (EXP), fed with the addition of 6% of extruded flaxseed mixture (contains 50% of extruded flaxseed; Croquelin, Walorex SAS, La Messaayais-35210 Combourtille, France). At the age of 45 days, 12 male (CONM & EXPM) and 12 female (CONF & EXPF) broilers from each group (4 from each replicate) were selected (by the average weight of the group ± 5%), marked, slaughtered, eviscerated and air-chilled. The carcasses were apportioned into cuts: back, two leg-thighs, two wings and breasts. Breasts (white meat) and leg-thighs (dark meat) were then deboned, the skin was removed and the meat was used for analysis. The experiment was carried out at the chicken farm of meat company “Union MZ”, Svilajnac, Serbia, and broilers were slaughtered in accordance with the Serbian national guidelines.

Cholesterol determination

Cholesterol determination in the meat was performed in the same manner as described by Milićević et al. (2014) as follows:

Cholesterol determination in the meat was performed after direct saponification (without prior lipid extraction) according to the method described by Maraschiello et al. (1996) and followed by the HPLC analysis; the data are expressed as mg per 100 g fresh meat. To ca. 100 mg of each homogenized broiler muscle, sample 2 ml of 0.5 M KOH in methanol was added and tubes were vortexed for 30 s. The mixture was directly saponified at 80 °C for 1 h. After cooling, 2 ml of distilled water, saturated with NaCl, were added. The tubes were vortexed for 30 s followed by the addition of 3 ml diethylether/hexane (1:1, v/v) and centrifuged for 10 min at 300 g. The upper phase was transferred to a clean tube and the ether/hexane extraction

step was repeated twice. All three extracts were combined and evaporated to dryness under a stream of nitrogen. The dry extracts were dissolved in 1,000 µl of mobile phase used for HPLC analysis and then immediately filtered: 10 µl was injected into HPLC. Cholesterol determination in the extract (from direct saponification) was performed using the HPLC/PDA system (Waters 2695 Separation module/Waters photodiode array detector, Milford, Ma., U.S.A.), on a Phenomenex Luna C 18 reverse/phase column, 150 mm x 3 mm, 5 µm particle size with C18 analytical guard column, 4 mm x 2 mm, at room temperature. The injected volume was 10 µl. The mobile phase was isopropanol-acetonitrile (20:80, v/v) at a flow rate of 1.2 ml·min^{-1}, isocratically. Detection was performed at 210 nm. Total analysis time was 10 min. Quantification of cholesterol was done by external standardization in a linear concentration range from 250–1,250 mg·kg$^{-1.}$ Recoveries of the spiked quantities ranged from 66.3 to 74.8%. Empower Pro software was used to control the HPLC system as well as for data acquisition and data processing.

Determination of fatty acids

Total lipids for fatty acids determination were extracted from the sample by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) with a mixture of n-hexane and isopropanol (60:40, v/v) as reported by Spiric et al. (2010). Total lipids were then converted to fatty acid methyl esters and the subsequent procedure was as described by Milićević et al. (2014), namely:

Total lipids were further converted to fatty acid methyl esters (FAMEs) by using 0.25 M trimethylsulfonium hydroxide (TMSH) in methanol (EN ISO 5509:2000, 2000). FAMEs were determined by capillary gas chromatography on GC Shimadzu 2010 (Kyoto, Japan) equipped with flame ionization detector and capillary HP-88 column (100 m x 0.25 mm x 0.2 µm, J&W Scientific, Ca., U.S.A.). The column temperature was programmed from the initial 125 ºC to final 230 ºC. Total analysis time was 50.5 min. The injector and detector temperatures were 250 ºC and 280 ºC, respectively. The carrier gas was nitrogen at a flow rate of 1.33 ml·min^{-1}. The injected volume was 1 µl and the injector split ratio 1:50. The individual fatty acids were identified by using the Supelco 37 Component FAME mix standard (Supelco, Bellefonte, Pa., U.S.A.).

Statistical analysis

The results were processed by a two-factorial analysis of variance (ANOVA). Tukey's HSD test was used to identify significant ($P<0.05$ and $P<0.01$) differences between groups. Calculations were done with software Statistica 6.0 (2001).

Results and discussion

Cholesterol content

Cholesterol content (Table 1) in white and dark meat was within the parameters stated by Ponte et al. (2008) and Dinh et al. (2011) between 46.13 and 52.94 mg per

100 g in white meat and 51.31–55.47 mg per 100 g in dark meat, although Komprda et al. (2003) found up to 80 mg per 100 g of cholesterol in leg meat.

The addition of extruded flaxseed to the diet of broilers did not affect the content of cholesterol in meat, which is in line with the results of Ajuyah et al. (1991) and Ayerza et al. (2002) who added flaxseed and chia seed, respectively, to the broiler diet.

Table 1. Means (±SD) of cholesterol content (mg·100 g^{-1}) of broiler meat fed with and without the addition of extruded flaxseed

	Control group (CON)		Experimental group (EXP)		P-values of model effects		
	Male	Female	Male	Female	Tmt	Gen	tmt*gen
White meat	46.13±6.26	51.57±5.1	46.21±5.32	52.94±4.01	NS	0.01	NS
Dark meat	51.31±7.12	55.47±3.65	51.58±7.58	55.41±3.58	NS	NS	NS

NS – not significant; Tmt – treatment; Gen – gender

Fatty acids content

Fatty acids content in white meat is presented in Table 2, and dark meat in Table 3. The main effects of the added extruded flaxseed to the broiler diet were the same in white and dark meat, namely a considerable increase in the content of α-linolenic acid (ALA, C18:3n-3), 1.5–3 times ($P<0.01$), and changes in the content of n-3 PUFA and the n-6/n-3 ratios. PUFA increased in the meat of the both EXP groups, compared to CON grpups. Unlike n-3 PUFA, the increase in n-6 PUFA was significant only with females ($P<0.05$). The n-6/n-3 ratio was considerably decreased in both genders (in dark meat $P<0.01$), while the PUFA/SFA ratio increased considerably only in the meat of females ($P<0.05$). The effect of the diet was significantly pronounced in dark meat, which has more fatty tissue, than in white meat, which is dominated by phospholipids, therefore the enrichment of breast meat with n-3 PUFA may be more difficult to achieve because the potential depot is generally smaller (Betti et al., 2009b). Gender had some influence on changes in the fatty acid profile of chicken meat (González-Esquerra and Leeson, 2001), which is also indicated by the results of this experiment because more pronounced changes in the fatty acid profile were detected in the meat (both white and dark) of females.

The dominant saturated fatty acids were palmitic (C16:0) and stearic acid (C18:0). Their contents, as well as the content of total saturated fatty acids (SFA) in white meat, were somewhat higher than in dark meat, therefore the PUFA/SFA ratio was poorer in white (0.49–0.75) than in dark meat (0.82–1.18). The addition of extruded flaxseed to the diet caused a reduction in the content of SFA, considerably only in the dark meat of females ($P<0.05$), while the PUFA/SFA ratio increased in both white and dark meat of females ($P<0.05$). Oleic acid (C18:1 cis-9) is always the prevailing

monounsaturated fatty acid (MUFA) (López-Ferrer et al., 2001b; Anjum et al., 2013), and its content is higher in meat than in the feed. López-Ferrer et al. (2001b) explain the high content of the oleic acid in meat by the fact that it originates in two ways: (i) it is directly deposited as a consequence of intake through feed; (ii) it is additionally synthesized in the liver and muscle tissue based on the intake of palmitic acid and stearic acid as a result of elongation and Δ-9 desaturation. The content of oleic acid was reduced in the dark meat in EXP of both genders ($P<0.05$), while as for white meat, this occurred only with females ($P<0.05$). With the addition of extruded flaxseed, MUFA content was significantly reduced in the dark meat of both genders ($P<0.05$), as well as in the white meat of females, probably due to the rise in the share of PUFA.

In nutritional and functional terms, PUFAs are the most important fatty acids in meat, and it is in terms of the content of total PUFA, n-6 and n-3 and individual PUFAs, that the largest variations can be found in literature. Although confusing at first, this fact is quite interesting as it confirms that nutritionally and functionally meat can be effectively influenced through animal diet. This can be illustrated with the data of Crespo and Esteve-Garcia (2002) who found that the value of n-6/n-3 in total body FA (g/100 g fat) of broilers fed by different feeds ranged from 0.6 to 49.6.

The prevalent PUFA was linoleic acid (LN, C18:2n-6). Its share in the white meat of CONF was considerably lower ($P<0.05$) relative to CONM, while the addition of extruded flaxseed in diet caused a significant increase in its content ($P<0.05$). The share of LN in dark meat is higher than in white meat, and the consequences of the flaxseed diet were the same as in the case of white meat with its content significantly increased in the meat of females ($P<0.05$). Kostadinović et al. (2016) also reported the prevalence of LN in total PUFA and its increase in white meat and decrease in dark meat.

The content of ALA (C18:3n-3) was considerably increased with the addition of extruded flaxseed to the diet. These findings correlate with those of Kostadinović et al. (2016) and Anjum et al. (2013) who also observed a significant increase ($P<0.05$) in ALA in breast and leg meat. In mammalians and poultry ALA is a potential precursor of eicosapetaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA) fatty acids (Betti et al., 2009b; Campioli et al., 2012); it is a dominant FA of the n-3 group and was associated with a lower risk of coronary heart disease (Nestel et al., 2015). EPA, DPA and DHA were not detected or their content was very low in the white and dark meat of the control and test groups, leaving no room for discussion or any definitive conclusions. These data conform with the statement by López-Ferrer et al. (2001b) that the conversion efficiency of ALA in long-chain PUFA is not high, i.e. that conversion is more intensive in the liver than in the muscles in which “the level is never nutritionally valuable” (López-Ferrer et al., 2001a). Komprda et al. (2013) also determined that the conversion efficiency of ALA in long chain PUFA was not high and that it was lower in diets richer in ALA. Betti et al. (2009b), using diets with 10% and 17% of ground flaxseed, reported significant increase of EPA and DPA after 35 days (in phospholipids and triacylglycerols fractions), but limited conversion of ALA into DHA in both fractions.

DOI: /10.5513/JCEA01/19.4.2050

Table 2. Fatty acids content (% total FA) in white meat

	Control group (CON)		Experimental group (EXP)		P-values of model effects		
	Male	Female	Male	Female	Tmt	Gen	Tmt*gen
C14:0	0.39±0.04^{a}	0.38±0.05^{a}	0.36±0.03^{a}	0.37±0.05^{a}	NS	NS	NS
C15:0	0.05±0.03^{a}	0.1±0.02^{b}	0.07±0.01^{ab}	0.09±0.02^{b}	NS	<0.001	NS
C16:0	26.06±1.55^{ab}	28.04±0.7^{a}	25.05±0.94^{b}	25.96±2.45^{ab}	0.025	0.035	NS
C16:1	4.41±0.46^{a}	3.85±0.73^{ab}	4.05±0.59^{a}	3.08±0.24^{b}	0.017	0.002	NS
C17:0	0.07±0.04^{a}	0.12±0.03^{b}	0.1±0.02^{ab}	0.14±0.02^{b}	NS	<0.001	NS
C18:0	9.54±0.39^{ab}	10.21±1.17^{a}	8.13±1^{b}	9.23±1.38^{ab}	0.011	NS	NS
C18:1cis-9	35.21±1.02^{A}	37.16±0.93^{B}	35.21±0.52^{A}	35.7±1.03^{a}	NS	0.003	NS
C18:2n-6	19.29±0.6^{a}	15.98±0.89^{b}	20.56±1.58^{a}	19.61±3.14^{a}	0.004	0.01	NS
C18:3n-6	0.18±0.07^{ac}	0^{b}	0.1±0.08^{c}	0.02±0.04^{b}	NS	<0.001	NS
C18:3n-3	1.62±0.2^{a}	0.72±0.16^{a}	3.03±0.75^{b}	2.2±0.62^{b}	<0.001	0.004	NS
C20:0	0^{a}	0.14±0.06^{b}	0^{a}	0.14±0.04^{b}	NS	<0.001	NS
C20:1	0.4±0.07^{a}	0.4±0.05^{a}	0.27±0.29^{a}	0.28±0.22^{a}	NS	NS	NS
C20:2	0.43±0.08^{a}	0.38±0.04^{a}	0.48±0.16^{a}	0.48±0.18^{a}	NS	NS	NS
C20:3n-6	0.43±0.26^{a}	0.25±0.15^{a}	0.46±0.11^{a}	0.29±0.08^{a}	NS	0.017	NS
C20:3n-3	0.92±0.54^{b}	1.8±0.27^{c}	0.3±0.37^{a}	1.67±0.32^{bc}	0.028	<0.001	NS
C22:1	1.17±0.76^{a}	0.48±0.56^{a}	1.17±0.45^{a}	0.71±0.3^{a}	NS	0.017	NS
C20:5n-3	0^{a}	0^{a}	0.05±0.04^{b}	0.02±0.03^{ab}	0.003	NS	NS
C22:5n-3	0.09±0.15^{ab}	0^{a}	0.23±0.21^{b}	0.01±0.03^{a}	NS	0.008	NS
C22:6n-3	0.01±0.01^{a}	0^{a}	0.03±0.06^{a}	0^{a}	NS	NS	NS
ΣSFA	36.11±1.77^{ab}	38.99±1.93^{A}	33.71±1.62^{B}	35.93±3.86^{ab}	0.013	0.02	NS
ΣMUFA	40.79±1.17^{ab}	41.49±1.53^{a}	40.43±0.6^{ab}	39.49±0.93^{b}	0.017	NS	NS
ΣPUFA	22.78±0.5^{b}	19.12±1.06^{A}	25.15±1.9^{B}	24.28±3.8^{B}	<0.001	0.02	NS
PUFA/SFA	0.63±0.04^{ab}	0.49±0.05^{A}	0.75±0.09^{B}	0.69±0.18^{b}	0.002	0.034	NS
Σn-3	2.63±0.49^{A}	2.52±0.26^{A}	3.65±0.7^{b}	3.91±0.78^{B}	<0.001	NS	NS
Σn-6	19.9±0.55^{a}	16.23±0.95^{B}	21.11±1.63^{A}	19.92±3.21^{a}	0.004	0.005	NS
n-6/n-3	7.78±1.54^{A}	6.5±0.73^{ab}	5.95±1.17^{b}	5.14±0.37^{B}	0.001	0.023	NS

$^{a, B}$ Values (mean±SD) within the same row with no common superscript differ significantly, P<0.05; $^{A, B}$ Values (mean±SD) within the same row with no common superscript differ significantly, P<0.01. NS – not significant; Tmt – treatment; Gen – gender

Table 3. Fatty acids content (% total FA) in dark meat

	Control group (CON)		Experimental group (EXP)		P-values of model effects		
	Male	Female	Male	Female	Tmt	Gen	Tmt*gen
C14:0	0.38±0.03^{c}	0.35±0.02^{bc}	0.33±0.02^{ab}	0.31±0.03^{a}	<0.001	0.023	NS
C15:0	0.05±0.01^{ab}	0.04±0^{a}	0.05±0^{b}	0.06±0^{c}	NS	<0.001	0.002
C16:0	23.19±0.86^{bc}	24.22±0.48^{c}	22.4±1.03^{ab}	21.24±1.48^{a}	<0.001	NS	0.016
C16:1	5.5±0.73^{a}	4.77±0.62^{a}	4.79±0.86^{a}	3.51±0.62^{b}	0.003	0.002	NS
C17:0	0.07±0.01^{a}	0.07±0.01^{a}	0.07±0^{a}	0.09±0.01^{b}	<0.001	0.004	0.004
C18:0	5.6±0.73^{a}	6.3±0.47^{a}	5.85±0.28^{a}	6.39±0.48^{a}	NS	0.008	NS
C18:1cis-9	37.32±1.23^{a}	37.9±0.93^{a}	34.88±1.69^{b}	34.78±1.43^{b}	<0.001	NS	NS
C18:2n-6	24.19±0.6^{a}	22.71±1.14^{a}	25.62±2.62^{ab}	27.26±2.37^{b}	<0.001	NS	NS
C18:3n-6	0.15±0.07^{a}	0.12±0.06^{a}	0.1±0.08^{a}	0.14±0.06^{a}	NS	NS	NS
C18:3n-3	1.98±0.25^{A}	1.75±0.31^{A}	4.47±0.58^{B}	4.66±0.35^{B}	<0.001	NS	NS
C20:0	0	0	0	0	-	-	-
C20:1	0.11±0.18^{AB}	0.32±0.25^{A}	0^{B}	0^{B}	0.002	NS	NS
C20:2	0.26±0.03^{a}	0.24±0.07^{a}	0.23±0.07^{a}	0.22±0.05^{a}	NS	NS	NS
C20:3n-6	0.54±0.07^{ab}	0.49±0.05^{a}	0.59±0.02^{b}	0.51±0.06^{a}	NS	0.005	NS
C20:3n-3	0.05±0.05^{a}	0.04±0.04^{a}	0.08±0.03^{a}	0.04±0.05^{a}	NS	NS	NS
C22:1	0.56±0.05^{a}	0.63±0.19^{a}	0.44±0.05^{a}	0.63±0.19^{a}	NS	NS	NS
C20:5n-3	0^{a}	0^{a}	0.02±0.02^{a}	0.02±0.03^{a}	NS	NS	NS
C22:5n-3	0.04±0.05^{a}	0.07±0.09^{a}	0.07±0.08^{a}	0.14±0.04^{a}	NS	NS	NS
C22:6n-3	0^{a}	0^{a}	0^{a}	0^{a}	NS	NS	NS
ΣSFA	29.28±1.45^{ab}	30.97±0.32^{a}	28.71±0.92^{b}	28.08±1.88^{b}	0.003	NS	0.039
ΣMUFA	43.39±1.89^{a}	43.29±1.1^{a}	40.1±2.41^{b}	38.91±2.02^{b}	<0.001	NS	NS
ΣPUFA	27.07±0.82^{a}	25.29±1.38^{a}	31.09±3.23^{b}	32.86±2.67^{b}	<0.001	NS	NS
PUFA/SFA	0.93±0.04^{ab}	0.82±0.05^{a}	1.09±0.15^{bc}	1.18±0.16^{c}	<0.001	NS	0.041
Σn-3	2.08±0.26^{A}	1.86±0.32^{A}	4.64±0.61^{B}	4.88±0.33^{B}	<0.001	NS	NS
Σn-6	24.87±0.61^{ab}	23.32±1.16^{a}	26.32±2.62^{ab}	27.91±2.39^{b}	<0.001	NS	NS
n-6/n-3	12.07±1.37^{A}	12.8±1.74^{A}	5.7±0.4^{B}	5.72±0.29^{B}	<0.001	NS	NS

$^{a, B}$ Values (mean±SD) within the same row with no common superscript differ significantly, P<0.05. $^{A, B}$ Values (mean±SD) within the same row with no common superscript differ significantly, P<0.01. NS – not significant; Tmt – treatment; Gen – gender

The considerable increase in ALA (in white meat, 87% and 205%, male and female respectively, and in dark meat, 126% and 166%) caused a significant increase in the content of n-3 FA in white and dark meat, as well as a more favorable n-6/n-3 ratio, which was very close to the recommended optimum of 4:1–5:1 (Gómez Candela et al., 2011) in both white and dark meat (both genders). Data in literature correspond to findings in this research and indicate that the inclusion of ALA (either as flaxseed or flaxseed oil) has a positive effect on the FA profile of meat. By adding 2% of flaxseed oil to the diet for 38 days, López-Ferrer et al. (2001a) recorded a reduction in n-6/n-3 from 6.11 to 1.69 in dark meat, whereas Rahimi et al. (2011), who added 7.5% of flaxseed for 42 days, established a reduction in n-6/n-3 from 26.06 to 6.92. Kostadinović et al. (2016) also reported a significant reduction ($P<0.05$) of the n-6/n-3 ratio in breast and leg meat of broilers fed with 2.5–10% extruded flaxseed enriched diet.

Conclusion

The addition of extruded flaxseed to the broiler diet affects the increase of the content of ALA, n-3 FA and PUFA, and leads to a reduction of the n-6/n-3 ratio in both genders and in both dark and white broiler meat. The influence was more pronounced with females because a considerable reduction of SFA and MUFA was observed, as well as an increase in the PUFA/SFA ratio, but also a significant increase in n-6 FA. These changes were observed in males; however (except in the case of MUFA in dark meat), no statistical significance was determined. A nutritionally important parameter n-6/n-3 was reduced (below 6) in both types of meat, coming down to values close to the recommended ones. Changes in the fatty acid composition were considerably more pronounced in dark meat.

Acknowledgment

The research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, project III–46009. The authors would also like to thank Mrs. Marija Stajić MA for assistance with translation and copy editing.

References

Ajuyah, A.O., Lee, K.H., Hardin R.T., Sim, J.S. (1991) Changes in the yield and in the fatty acid composition of whole carcass and selected meat portions of broiler chickens fed full-fat oil seeds. Poultry Science, 70 (11), 2304–2314. DOI: https://doi.org/10.3382/ps.0702304

Anjum, F.M., Haider, M.F., Khan, M.I., Sohaib, M., Arshad, M.S. (2013) Impact of extruded flaxseed meal supplemented diet on growth performance, oxidative stability and quality of broiler meat and meat products. Lipids Health and Disease, 12 (1), 13. DOI: https://doi.org/10.1186/1476-511X-12-13

Ayerza, R., Coates, W., Lauria, M. (2002) Chia seed (Salvia hispanica L.) as an omega-3 fatty acid source for broilers: influence on fatty acid composition, cholesterol and fat content of white and dark meats, growth performance, and sensory characteristics. Poultry Science, 81 (6), 826–837. DOI: https://doi.org/10.1093/ps/81.6.826

Betti, M., Schneider, B.L., Wismer, W.V., Carney, V.L., Zuidhof, M.J., Renema, R.A. (2009a) Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. Poultry Science, 88 (5), 1085–1095. DOI: https://doi.org/10.3382/ps.2008-00158

Betti, M., Perez, T.I., Zuidhof, M.J., Renema, R.A. (2009b) Omega–3 enriched broiler meat, 3 fatty acid distribution between triacylglycerol and phospholipid classes. Poultry Science, 88 (8), 1740–1754. DOI: https://doi.org/10.3382/ps.2008-00449

Bou, R., Codony, R., Tres, A., Decker E.A., Guardiola, F. (2009) Dietary strategies to improve nutritional value, oxidative stability, and sensory properties of poultry products. Critical Reviews in Food Science and Nutrition, 49 (9), 800–822. DOI: https://doi.org/10.1080/10408390902911108

Campioli, E., Rustichelli, C., Avallone, R. (2012) n-3 Dietary supplementation and lipid metabolism: Differences between vegetable- and fish-derived oils. Journal of Functional Foods, 4 (1), 207–212. DOI: https://doi.org/10.1016/j.jff.2011.10.006

Crespo, N., Esteve-Garcia, E. (2002) Nutrient and fatty acid deposition in broilers fed different dietary fatty acid profiles. Poultry Science, 81 (10), 1533–1542. DOI: https://doi.org/10.1093/ps/81.10.1533

Dinh, T.T.N., Thompson, L.D., Galyean, M.L., Brooks, J.C., Patterson, K.Y., Boylan, L.M. (2011) Cholesterol content and methods for cholesterol determination in meat and poultry. Comprehensive Reviews in Food Science and Food Safety, 10 (5), 269–289. DOI: https://doi.org/10.1111/j.1541-4337.2011.00158.x

EN ISO 5509:2000 (2000) Animal and vegetable fats and oils - Preparation of methyl esters of fatty acids. Geneva: International Organization for Standardization.

Food and Agriculture Organization (2013) FAO Statistical yearbook 2013: World food and agriculture. Rome: Food and Agriculture Organization of the United Nations.

Gómez Candela, C., Bermejo López, L.M., Loria Kohen, V. (2011) Importance of a balanced omega 6/omega 3 ratio for the maintenance of health: Nutritional recommendations. Nutrición Hospitalaria, 26 (2), 323–329.

González-Esquerra, R., Leeson, S. (2001) Alternatives for enrichment of eggs and chicken meat with omega-3 fatty acids. Canadian Journal of Animal Science, 81 (3), 295–305. DOI: https://doi.org/10.4141/A00-092

Hathwar, S., Rai, A., Modi, V., Narayan, B. (2012) Characteristics and consumer acceptance of healthier meat and meat product formulations—a review. Journal Food Science and Technology, 49 (6), 653–664. DOI: https://doi.org/10.1007/s13197-011-0476-z

Komprda, T., Zelenka, J., Fajmonová, E., Bakaj, P., Pechová, P. (2003) Cholesterol content in meat of some poultry and fish species as influenced by live weight and total lipid content. Journal of Agricultural and Food Chemistry, 51 (26), 7692–7697. DOI: https://doi.org/10.1021/jf030378r

Komprda, T., Zorníková, G., Rozíková, V., Borkovcová, M., Przywarová, A. (2013) The effect of dietary Salvia hispanica seed on the content of n-3 long-chain polyunsaturated fatty acids in tissues of selected animal species, including edible insects. Journal of Food Composition and Analysis, 32 (1), 36–43. DOI: https://doi.org/10.1016/j.jfca.2013.06.010

Kostadinović, Lj., Popović, S., Čolović, D., Vukmirović, Đ., Tasić, T., Puvača, N. Lević, J. (2016) Effect of extruded flaxseed in broiler diets on blood oxidative stability and meat fatty acid composition. European Poultry Science, 80 (1), 1–14. DOI: https://doi.org/10.1399/eps.2016.140

López-Ferrer, S., Baucells, M.D., Barroeta, A.C., Galobart, J., Grashorn, M.A. (2001a) 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: https://doi.org/10.1093/ps/80.6.753

López-Ferrer, S., Baucells, M.D., Barroeta, A.C., Grashorn, M.A. (2001b) n-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. Poultry Science, 80 (6), 741–752. DOI: https://doi.org/10.1093/ps/80.6.741

Maraschiello, C., Díaz, I., Regueiro, J.A.G. (1996) Determination of cholesterol in fat and muscle of pig by HPLC and capillary gas chromatography with solvent venting injection. Journal of High Resolution Chromatography, 19 (3), 165–168. DOI: https://doi.org/10.1002/jhrc.1240190309

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 Disease, 13, 42. DOI: https://doi.org/10.1186/1476-511X-13-42

Nestel, P., Clifton, P., Colquhoun, D., Noakes, M., Mori, T.A., Sullivan, D. Thomas, B. (2015) Indications for Omega-3 long chain polyunsaturated fatty acid in the prevention and treatment of cardiovascular disease. Heart, Lung and Circulation, 24 (8), 769–779. DOI: https://doi.org/10.1016/j.hlc.2015.03.020

Panda, A.K., Sridhar, K., Lavanya, G., Prakash, B., Rao, S.V.R., Raju, M.V.L.N. (2015) Growth performance, carcass characteristics, fatty acid composition and sensory attributes of meat of broiler chickens fed diet incorporated with linseed oil. Indian Journal of Animal Science, 85 (12), 74–77.

Ponte, P.I.P., Alves, S.P., Bessa, R.J.B., Ferreira, L.M.A., Gama, L.T., Brás, J.L.A., Fontes, C.M.G.A., Prates, J.A.M. (2008) Influence of pasture intake on the fatty acid composition, and cholesterol, tocopherols, and tocotrienols content in meat from free-range broilers. Poultry Science, 87 (1), 80–88. DOI: https://doi.org/10.3382/ps.2007-00148

Rahimi, S., Kamran Azad, S. Karimi Torshizi, M.A. (2011) Omega-3 enrichment of broiler meat by using two oil seeds. Journal of Agricultural Science and Technology, 13 (3), 353–365.

Shafey, T.M., Mahmoud, A.H., Hussein, E.-S., Suliman, G. (2014) The performance and characteristics of carcass and breast meat of broiler chickens fed diets containing flaxseed meal. Italian Journal of Animal Science, 13, 3514. DOI: https://doi.org/10.4081/ijas.2014.3514

Spiric, A., Trbovic, D., Vranic, D., Djinovic, J., Petronijevic, R., Matekalo-Sverak, V. (2010) Statistical evaluation of fatty acid profile and cholesterol content in fish (common carp) lipids obtained by different sample preparation procedures. Analytica Chimica Acta, 672 (1–2), 66–71. DOI: https://doi.org/10.1016/j.aca.2010.04.052

Živković, D., Stajić, S., Stanišić, N. (2013) Possibilities for the production of meat and meat products with improved nutritional and functional value. In: Pavlovski, Z., Proceedings of the 10th International symposium modern trends in livestock production. Belgrade, Serbia, 2–4 October 2013, Institute for Animal Husbandry, 188–199.

Živković, D., Lilić, S., Stajić, S., Vranić, D., Trbović, D., Stanišić, N. (2017) Effect of extruded flaxseed enriched diet on physico-chemical and sensory characteristics of broiler meat. Biotechnology in Animal Husbandry, 33 (2), 221–231. DOI: https://doi.org/10.2298/BAH1702221Z