



Difference in fatty acid composition and related nutritional indices of meat between two lines of slow-growing chickens slaughtered at different ages

Teodora Popova¹, Maya Ignatova¹, Evgeni Petkov¹, and Nikola Stanišić²

¹Institute of Animal Science, 2232 Kostinbrod, Bulgaria

²Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Republic of Serbia

Correspondence to: Teodora Popova (tlpopova@yahoo.com)

Received: 6 April 2016 – Revised: 14 June 2016 – Accepted: 23 June 2016 – Published: 12 July 2016

Abstract. The fatty acid profile and the related indices of the nutritional quality of breast and thigh muscles were studied in two lines of chickens – La Belle (LB) and White Plymouth Rock (WPR) – slaughtered at the age of 9 and 18 weeks. The fatty acid profile was more affected by the age than the line of the birds; however, the influence of both differed between the breast and thigh. The content of total saturated fatty acids (SFAs) increased in the thigh ($P < 0.01$), while that of the monounsaturated fatty acids (MUFAs) decreased with age in both muscles ($P < 0.001$). This corresponded to the significant decrease in C18:1 in the older chickens and the lower desaturase activity ($P < 0.001$). The contents of C20:4n-6, C22:5n-3 and C22:6n-3 and the total amount of polyunsaturated fatty acids (PUFAs) in breast were higher ($P < 0.001$) at the age of 18 weeks. A similar pattern in the individual and total PUFA was observed in the thighs. The effect of line was more visible in the breast, leading to a lower C14:0 content and C20:5n-3 and a higher C18:0 content in the WPR chickens ($P < 0.001$), corresponding to the higher elongase and thioesterase indices in these birds. Both atherogenic (AI) and thrombogenic (TI) indices were lowered, while the ratio of hypocholesterolemic / hypercholesterolemic fatty acids (h / H) and polyunsaturated / saturated fatty acids (P / S) increased in the breast of the birds at 18 weeks. In breast and thigh meat, the ratio of n-6 / n-3 PUFA decreased in the older chickens ($P < 0.001$).

1 Introduction

The interest in slow-growing chickens has been increasing recently due to consumers' demands for healthier food (Fanatico et al., 2007). Although these birds generally have less efficient growth performance, they are well adapted to alternative rearing systems (Fanatico et al., 2005, 2006) and show superior meat quality with higher protein and lower fat content in comparison to the fast growing broilers (Poltowicz and Doktor, 2012). As stated by Dal Bosco et al. (2011), some slow-growing poultry products have a long history in Europe as for example the French Label Rouge program, which requires outdoor access and a growing period of at least 81 days for the birds. It occupies a significant part of the French poultry market despite selling products for a higher price compared to the conventional poultry products (Westgren, 1999; Fanatico and Born, 2001). However, in Bulgaria

commercial rearing of slow-growing chickens is limited and the research on the meat quality of such breed lines is still scarce. As lines of the national gene pool, until recently La Belle and White Plymouth Rock were not of interest for broiler production. With the increasing importance of slow-growing chicken production, possibilities appear for these lines to be used to create crosses with slower growth, no deposition of abdominal fat and a high quality of meat (Petkov et al., 2013; Petkov, 2015). This, however, makes it necessary to study the influence of different factors, such as genetic line and age on various aspects of meat quality in order to further improve it. Fatty acid composition is a very important component of meat quality and has received considerable interest in view of its implications for human health (De Smet et al., 2004). It is well known that the higher intake of saturated fatty acid (SFA) in the human diet increases the risk of the development of coronary heart disease, atherosclerosis and can-

cer (Mensink and Katan, 1992), whereas monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), especially n-3, have a number of associated health benefits (Kris-Etherton, 1999; Siriwardhana et al., 2012). Foods rich in long-chain n-3 PUFA have been shown to improve cardiovascular health (Albert et al., 2002; Raitt et al., 2005; Harris, 2007; Lu et al., 2011), and reduce inflammation (Ferrucci et al., 2006; Calder, 2008; Fetterman and Zdanowicz, 2009; Wortman et al., 2009; Figueras et al., 2011) and behavioral disorders, such as depression (Rondanelli et al., 2011).

The lipid composition of meat is mostly affected by the nutrition of the birds, but other factors such as age and breed line also contribute to the changes in the fatty acid profile. However, in the available literature, the data on dependencies of the fatty acid composition on these two factors remain relatively scarce. Komprda et al. (2000) found a significant effect of age on the content of the total SFA, MUFA and PUFA in two chicken genotypes with different growth potential. Díaz et al. (2012) reported differences in the fatty acid composition between capons of indigenous and commercial strains at different ages, finding that the age effect was more important than that of the breed. In other research, Dal Bosco et al. (2012) also reported a different fatty acid deposition in various genotypes of chickens reared under an organic system; however, this was associated with differences in behavior and grass consumption.

This study is the first of its kind in Bulgaria, aiming to assess the potential of lines, representative of the national gene reserve, to produce slow-growing chickens with a high quality of meat in regard to its lipid profile, as affected by the line and age of the birds. The precise evaluation of the influence of these factors on the fatty acids and related indices of the nutritional quality of meat requires first experiments under controlled conditions in a conventional system which will serve as a basis for further investigation of alternative systems.

2 Material and methods

2.1 Experimental animals, slaughtering and sampling

The experiment was carried out in the experimental poultry farm of the Institute of Animal Science–Kostinbrod, Bulgaria, with a total of 158 male La Belle (LB) and 239 White Plymouth Rock (WPR) chickens, which were obtained from the parent stocks kept in the Institute. The 1-day-old chickens were placed in a deep litter facility with a stocking density of 14 birds m^{-2} in separate pens in the same poultry house. All the birds were fed a starter, grower and finisher diet (Table 1) ad libitum, which was evaluated according to AOAC (2004). Water was provided ad libitum with a nipple waterer. The lighting regime was 15 h of light and 9 h of darkness, and the temperature ranged between 20 and 24 °C. During rearing, the individual body weight of the chickens and their feed intake was recorded every week and the number of dead

Table 1. Diet composition

Item, %	Starter	Grower	Finisher
Corn	32.34	33.29	34.54
Wheat	30.00	30.00	30.00
Soybean meal	27.00	19.50	15.50
Sunflower meal	5.00	10.00	13.00
Sunflower oil	2.00	3.80	4.00
Oxiguard	0.01	0.01	0.01
Limestone	1.30	1.20	1.10
Monocalcium diphosphate	0.80	0.75	0.60
Salt	0.25	0.20	–
Sodium bicarbonate	0.30	0.25	–
Vitamin–mineral premix	1.00	1.00	1.25
Analyzed chemical composition, %			
Protein	20.52	18.95	18.07
Fat	4.31	6.09	6.33
Crude fiber	4.27	4.92	5.38
Ashes	5.31	5.01	4.67
NNC ¹	54.45	54.03	54.55
ME ² , kcal kg^{-1}	2952.89	3045.20	3052.59

¹ NNC: non-nitrogen extractive compounds. ² ME: metabolizable energy.

birds every day. At the age of 9 and 18 weeks, 6 chickens of each line were selected for slaughter based on the average live weight (1.258 ± 0.05 kg for La Belle and 1.045 ± 0.07 kg for the White Plymouth Rock line at 9 weeks; 2.778 ± 0.11 and 2.185 ± 0.10 kg for the 18-week-old La Belle and White Plymouth Rock, respectively). After stunning, decapitation and bleeding, the carcasses were plucked, eviscerated and stored at 4 °C for 24 h. Neck, legs and edible viscera (heart, liver, gizzard) were removed in order to obtain the ready-to-cook carcass. Furthermore, the breast and thigh muscles of each chicken were separated and minced with a meat grinder, and samples for the determination of the fatty acid profile of the muscles (10 g) were taken, vacuum-packed and stored at –20 °C until analysis.

2.2 Fatty acid analysis

Total lipids from the feed and the breast and thigh muscles were extracted according to the method of Bligh and Dyer (1959). Methyl esters of the total lipids, isolated by preparative thin layer chromatography, were obtained using 0.01 % solution of sulfuric acid in dry methanol for 14 h, as described by Christie (1973). The fatty acid composition of total lipids was determined by gas–liquid chromatography (GLC) analysis using a chromatograph C Si 200 equipped with a capillary column (DM-2330: 30 m \times 0.25 mm \times 0.20 μ m) and hydrogen as a carrier gas. The oven temperature was first set to 160 °C for 0.2 min, then raised until 220 °C at a rate of 5° min^{-1} and then held

Table 2. Fatty acid composition (% of total FAME) of the diet.

Fatty acid	Starter	Grower	Finisher
C14:0	0.20	0.21	0.17
C16:0	12.30	20.97	17.83
C16:1	0.28	0.19	0.17
C18:0	4.93	9.55	6.81
C18:1	28.28	49.34	57.00
C18:2n-6	52.76	19.59	17.83
C18:3n-3	1.25	0.15	0.19
SFA	17.43	30.73	24.81
MUFA	28.56	49.53	57.17
PUFA	54.01	19.74	18.02

for 5 min. The temperatures of the detector and injector were 230 °C. Methyl esters were identified through a comparison to the retention times of the standards. Fatty acids are presented as percentages of the total amount of the methyl esters (FAME) identified (Christie, 1973).

The $\Delta 9$ -desaturase index, as an indirect index of stearoyl-CoA desaturase (SCD) activity, was calculated as C18:1 / C18:0 (SCD18) or C16:1 / C16:0 (SCD16) (Lee et al., 1996). The elongase index was calculated as the ratio of C18:0 to C16:0, whereas the thioesterase index was calculated as the ratio of C16:0 to myristic acid C14:0 (Zhang et al., 2007). The amount of each fatty acid was used to calculate the indices of atherogenicity and thrombogenicity, as proposed by Ulbricht and Southgate (1991):

$$AI = (4 \times C14:0 + C16:0) / [MUFA + \Sigma(n-6) + \Sigma(n-3)];$$

$$TI = (C14:0 + C16:0 + C18:0) / [0.5 \times MUFA + 0.5 \times (n-6) + 3 \times (n-3) + (n-3) / (n-6)].$$

The h / H ratio was calculated, as suggested by Santos-Silva et al. (2002):

$$h / H = (C18:1 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0).$$

2.3 Statistical analysis

Data were statistically evaluated by two-way ANOVA as the line of the birds, age and their interaction were included in the model. The JMP v.7 software package was used to perform the statistical analysis (JMP Version 7, SAS Institute Inc. Cary, NC).

3 Results and discussion

The fatty acid composition of the starter, grower and finisher diets is presented in Table 2. Mono- and polyunsaturated fatty acids represented the major classes of the fatty acids in

the three diets. Oleic acid (C18:1) was the main monounsaturated fatty acid, forming 28.28 % in the starter diet, while its content in the grower and finisher diets increased substantially to 49.34 and 57 %, respectively. The most abundant of the PUFAs was linoleic acid (C18:2n-6), which had the greatest proportion in the starter feed: 52.76 %. In grower and finisher diets, its contents were 19.59 and 17.83 %. Palmitic acid (C16:0) has the largest proportion of the SFAs: 12.30, 20.97 and 17.83 %, respectively, for the starter, grower and finisher diets.

The fatty acid composition of the breast and thigh are presented in Table 3. The major saturated fatty acids in both muscles are palmitic (C16:0) and stearic (C18:0). The content of C16:0 in the breast was significantly affected by age, leading to its decrease in the older chickens ($P < 0.01$) whereas the proportion of C18:0 in both breast and thigh increased with advancing age ($P < 0.001$). While no effect of the line was observed in regard to C16:0 in the muscles, the content of C18:0 in the breast significantly differed between LB and WPR chickens, showing higher content in the latter ($P < 0.001$). The third identified SFA in this study was myristic acid (C14:0). Its content was affected by both line ($P < 0.001$) and age ($P < 0.01$) in the breast of the chickens. Higher content of C14:0 was observed in LB than WPR chickens, while in both lines, its proportion decreased with age. Bellizzi et al. (1994) found a very strong relation of C14:0 with coronary heart disease, and according to Fernandez and West (2005) this fatty acid is considered hypercholesterolemic. This is due to repression of the hepatic low-density lipoprotein (LDL) receptor synthesis as well as direct stimulation of hepatic LDL synthesis. When present in the human diet, C16:0 has also been associated with an increase in plasmatic cholesterol concentration; however, this association is stronger for C14:0 which has the potential to increase the cholesterol to 5 to 6 times greater than C16:0 (Yu et al., 1995). On the other hand, C18:0 has a neutral effect on the concentration of total serum cholesterol, including no apparent impact on either LDL or high-density lipoprotein (HDL; Kris-Etherton et al., 2005; Mensink, 2005). The total content of SFA in breast remained unaffected by the line and the age of the birds; however, in thigh it was increased significantly in the older birds ($P < 0.01$), reflecting the changes in C18:0.

Slaughtering age had a significant effect on the major monounsaturated fatty acid C18:1 in the breast ($P < 0.01$) and thigh ($P < 0.001$) of the chickens, showing a decrease in its content in the older birds. Similarly, differences between the ages of the birds were observed in regard to the content of C16:1; however, significant interaction of this factor with the line was found in both breast and thigh. As a whole, LB chickens displayed a considerable decrease in C16:1 with age, while in WPR, its percentage remained approximately the same in both muscles. The differences in the proportion of C16:1 in the muscles between the two lines were more substantial at the age of 9 weeks, with LB displaying higher contents of this fatty acid compared to WPR.

Table 3. Fatty acid composition (% of total FAME) of the breast and thigh in La Belle (LB) and White Plymouth Rock (WPR) chickens according to the line and age.

Fatty acid	LB		WPR		SE ¹	Significance of the factors		
	9 w	18 w	9 w	18 w		Line	Age	Line × age
Breast								
C14:0	0.86	0.67	0.61	0.54	0.08	***	**	NS
C16:0	29.37	26.81	28.93	25.74	1.68	NS	**	NS
C18:0	9.50	11.87	11.51	13.37	0.89	***	***	NS
SFA	39.73	39.35	41.05	39.65	1.95	NS	NS	NS
C16:1	4.24	1.96	2.62	2.12	0.71	*	***	**
C18:1	25.15	19.87	23.86	21.79	3.20	NS	**	NS
MUFA	29.39	21.83	26.48	23.91	3.68	NS	***	NS
C18:2n-6	19.77	18.68	21.12	17.29	1.13	NS	***	**
C18:3n-3	0.43	0.47	0.42	0.40	0.10	NS	NS	NS
C20:2n-6	0.39	0.38	0.48	0.39	0.08	NS	NS	NS
C20:3n-6	0.87	0.77	0.95	1.00	0.19	NS	NS	NS
C20:4n-6	7.54	15.10	7.86	13.81	2.50	NS	***	NS
C20:5n-3	0.47	0.36	0.25	0.21	0.11	***	NS	NS
C22:5n-3	0.74	1.75	0.70	1.82	0.33	NS	***	NS
C22:6n-3	0.67	1.31	0.69	1.52	0.35	NS	***	NS
PUFA	30.88	38.82	32.47	36.44	3.28	NS	***	NS
Thigh								
C14:0	0.68	0.62	0.69	0.71	0.09	NS	NS	NS
C16:0	23.93	22.30	23.16	24.84	1.99	NS	NS	NS
C18:0	10.04	14.82	11.91	13.94	1.63	NS	***	NS
SFA	34.65	37.74	35.76	39.49	2.18	NS	**	NS
C16:1	5.19	2.69	3.63	3.67	1.10	NS	*	*
C18:1	31.83	22.31	27.92	23.32	3.14	NS	***	NS
MUFA	37.02	25.00	31.55	26.99	3.67	NS	***	*
C18:2n-6	21.19	23.62	24.30	21.23	1.40	NS	NS	***
C18:3n-3	0.56	0.63	0.61	0.67	0.10	NS	NS	NS
C20:2n-6	0.29	0.38	0.40	0.32	0.06	NS	NS	**
C20:3n-6	0.42	0.40	0.48	0.43	0.07	NS	NS	NS
C20:4n-6	5.04	10.14	5.90	8.96	1.64	NS	***	NS
C20:5n-3	0.03	0.22	0.03	0.07	0.06	**	***	**
C22:5n-3	0.45	1.08	0.51	1.04	0.13	NS	***	NS
C22:6n-3	0.35	0.79	0.46	0.80	0.15	NS	***	NS
PUFA	28.33	37.26	32.69	33.52	2.87	NS	***	*

¹ SE: standard error. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. NS: not significant.

The total content of MUFA in the breast decreased with age ($P < 0.001$), while in the thighs, besides their lower content in the older chickens, the age and breed line interacted significantly ($P < 0.05$). The lower concentration of MUFA in the chickens at 18 weeks of age, mainly at the expense of the decreased proportion of C18:1 could be explained with the lower total lipids in these birds, which we reported in another paper (Popova et al., 2016). Raes et al. (2001) stated that the proportions of C18:1 diminished with a decrease in the total intramuscular lipid content in beef. However, a similar relationship between the contents of C18:1 and the lipid content is not always observed in poultry (Michalczuk et al., 2016).

It is difficult to discriminate between the influence of the age and line of the birds on the contents of C18:2n-6. In both breast and thigh significant interaction between the factors was observed. In the breast, however, a decrease was displayed in LB and WPR chickens at a later age. No significant difference due to age or line was observed in the percentage of C18:3n-3. In their study on three genetic lines of chickens, Dal Bosco et al. (2014a) did not observe changes in the content of C18:2 in breast with age, while the proportion of C18:3n-3 increased only in one line.

Of all the PUFA, the genetic line affected only the proportion of C20:5n-3, which in the breast was significantly higher in LB ($P < 0.001$) than WPR. Difference between the lines

Table 4. Indices of lipid metabolism in the breast and thigh meat in La Belle (LB) and White Plymouth Rock (WPR) chickens according to the line and age.

Item	LB		WPR		SE ¹	Significance of the factors		
	9 w	18 w	9 w	18 w		Line	Age	Line × age
Breast								
Elongase	0.32	0.44	0.40	0.52	0.04	***	***	NS
Thioesterase	34.55	40.33	47.50	49.85	6.95	**	NS	NS
SCD16	0.14	0.07	0.09	0.08	0.02	*	***	**
SCD18	2.67	1.69	2.11	1.64	0.42	NS	***	NS
Thigh								
Elongase	0.42	0.66	0.52	0.56	0.09	NS	**	*
Thioesterase	35.89	36.09	33.71	34.98	3.11	NS	NS	NS
SCD16	0.21	0.12	0.15	0.15	0.04	NS	**	*
SCD18	3.22	1.57	2.38	1.69	0.48	NS	***	*

¹ SE: standard error. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. NS: not significant.

in regard to the content of this fatty acid existed in thighs as well ($P < 0.01$); however, a strong interaction with age was detected ($P < 0.01$). The contents of C20:4n-6, C22:5n-3 and C22:6n-3 were affected by age in the breast and thigh of the chickens from both lines ($P < 0.001$), showing a considerable increase in the older birds. In a previous study with male layer-type chickens (Popova et al., 2015), we found a substantial increase in C20:4n-6, C22:5n-3 and C22:6n-3 in the breast of the birds slaughtered at 12 weeks of age compared to 5 weeks, which coincides with the results presented here. In line with our results, Dal Bosco et al. (2014a) found a substantial increase in the n-3 PUFA in the birds at 81 days, compared to 70 days of age. Muscles contain significant amounts of long-chain PUFAs, which are mainly formed in the liver from C18:2n-6 and C18:3n-3 by the action of $\Delta 5$ - and $\Delta 6$ -desaturase and elongase enzymes (Schmitz and Ecker, 2008; Wood et al., 2008). The significantly higher content of the long-chain PUFA in the birds slaughtered at 18 weeks when compared to the 9-week-olds, suggests increased activity of the abovementioned enzymes with advancing age. On the other hand, the higher proportions of the individual PUFA and particularly C20:4n-6 in the breast and thigh in the older chickens corresponds to the lower content of C18:1. Høstmark and Haug (2013) found a strong inverse relationship between the percentage of C18:1 and C20:4n-6 in chicken breast. According to these authors, C18:1 acts as an inhibitor for the $\Delta 5$ - and $\Delta 6$ -desaturase and/or 5-elongase systems, while at the same time C20:4n-6 might inhibit the SCD.

The strong influence of age on the proportion of the individual fatty acids in the breast was reflected also in the proportion of the total PUFA, which was increased ($P < 0.001$) in the chickens at the age of 18 weeks compared to the 9-week-old birds. An increase in the total content of PUFA was also displayed in thigh ($P < 0.001$), more substantial in the slower-growing LB chickens, compared to WPR. Sim-

ilarly to us, Dal Bosco et al. (2014a) reported higher contents of total PUFA and the long-chain derivatives in older birds. On the other hand, Michalczuk et al. (2016) observed changes in the PUFA with age, depending on the genotype, as in the fast- and medium-growing chickens, the total PUFA increased from 5 to 7 weeks, while in a slow-growing line no changes were presented.

In line with the abovementioned results some estimated indices of the lipid metabolism (Table 4) confirm that the fatty acid composition could depend on the age, line and also their interaction; however, the effect differs in the breast and thigh of the chickens. Elongase activity, described as the ratio C18:0 / C16:0, showed significantly higher values in the breast of WPR chickens ($P < 0.001$) and also increased with age in both lines ($P < 0.001$). In the thigh, the differences were attributed mainly to the age of the birds, showing an increase in the older ones. It was more pronounced in LB, while the changes in WPR were minor, which was illustrated by the significant interaction between the factors. The changes in the elongase activity in the aged birds corresponded to those of the content of C16:0 and C18:0 described above and also could be explained with the decrease in the intramuscular total lipid content. In fact, Kazala et al. (1999) observed a negative relationship between the intramuscular fat content and the elongase activity in beef. Our results are in line with Poureslami et al. (2010), who reported a significant increase in elongase with age in broilers. In a study on the fatty acid profile and lipid metabolism, Dal Bosco et al. (2012) reported a significant effect of the genotype of the birds on the elongase activity. The authors observed higher elongase activity in slower-growing genotypes compared to medium- and fast-growing types, which contradicts to our results, regarding the genotype of the birds.

Thioesterase in the fatty acid synthase complex is responsible for terminating the cycles of fatty acid synthesis and

Table 5. Fatty acid indices in breast and thighs in La Belle (LB) and White Plymouth Rock (WPR) chickens according to the line and age.

Item	LB		WPR		SE ¹	Significance of the factors		
	9 w	18 w	9 w	18 w		Line	Age	Line × age
Breast								
P / S	0.77	0.98	0.79	0.92	0.09	NS	***	NS
n-6 / n-3	12.69	9.08	15.01	8.48	1.43	NS	***	*
AI	0.54	0.48	0.53	0.46	0.04	NS	**	NS
TI	1.10	0.98	1.18	0.99	0.10	NS	***	NS
h / H	1.81	2.09	1.86	2.18	0.20	NS	**	NS
Thigh								
P / S	0.82	0.99	0.92	0.85	0.08	NS	NS	*
n-6 / n-3	19.61	12.76	19.50	12.16	1.42	NS	***	NS
AI	0.41	0.40	0.40	0.46	0.04	NS	NS	NS
TI	0.96	0.99	0.98	1.08	0.09	NS	NS	NS
h / H	2.44	2.57	2.52	2.22	0.29	NS	NS	NS

¹ SE: standard error. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. NS: not significant.

releasing the newly synthesized fatty acid. The enzyme has both C14-acyl acyl carrier protein (ACP) and C16-acyl ACP as substrates, as C16:0 is the major product. The ratio of C16:0 to C14:0 was utilized to reflect the selective cleavage of thioesterase on C14-acyl ACP or C16-acyl ACP because the greater the thioesterase index, the less cleavage there is of C14-acyl ACP. In the present study thioesterase in the breast was significantly affected by the line of the birds ($P < 0.01$), as WPR displayed higher activity. This corresponds to the lower content of C14:0 in these birds. In line with our results, thioesterase activity has been reported to depend on the genotype in birds (Dal Bosco et al., 2012), pigs (Martino et al., 2014) and also in rabbits (Dal Bosco et al., 2014b).

Stearoyl-CoA desaturase catalyzes the introduction of the first cis-double bond in the Δ -9 position (between carbon 9 and 10) of saturated fatty acids, and the preferred substrates are palmitoyl- and stearoyl-CoA (Ntambi, 1999). Significant interaction of the age and line was observed in regard to the SCD16 in breast ($P < 0.01$) and thigh ($P < 0.05$). This was associated mainly with the differences in the content of C16:1 between the lines at 9 weeks and the considerable age effect in the LB chickens. The SCD18 index was affected by the age of the birds in both breast and thigh ($P < 0.001$) and decreased in the 18-week-old chickens. This corresponds to the substantial increase in the content of n-6 PUFA with age since according to Choi et al. (2001), SCD expression can be markedly suppressed by C18:2n-6, C18:3n-3 and C20:4n-6.

The ratios of P / S, n-6 / n-3 PUFA, h / H, and atherogenic and thrombogenic indices are widely used to evaluate the nutritional value of fat. The ratio between PUFA and SFA in the breast was significantly affected by the age of the chickens ($P < 0.001$) and was higher in the older birds (Table 5). It corresponds to the pattern of changes in the PUFA content and increased from 0.77 to 0.98 in LB and 0.79–0.92

in WPR. In the thigh muscle, however, both line and age interacted significantly ($P < 0.05$) in regard to this trait. In breast and thighs, the ratio between n-6 and n-3 PUFA exhibited lower values in the older chickens ($P < 0.001$). In both lines, at both ages of slaughter, the P / S ratio was higher than the minimum recommended value of 0.4 (Enser et al., 2001). On the other hand, the values of n-6 / n-3 were relatively high – above the recommended values of 4. It could be suggested that such high values of this ratio are associated with the conventional rearing of the birds and the high content of n-6 PUFA, mainly C18:2n-6, in the feed compared to grass, where C18:3n-3 is predominant. However, Dal Bosco et al. (2012) reported values of n-6 / n-3 ranging from 6.23 to 17.9 in different chicken genotypes reared under an organic system. On the other hand, in the older birds, n-6 / n-3 ratio decreased by, on average, 36.6% in the breast and thigh, which suggests the positive influence of the later age at slaughter on this trait and especially on the increase in the n-3 PUFA, which is desirable in foods of animal origin.

In an attempt to take into account the different effects of the various fatty acids, Ulbricht and Southgate (1991) proposed two indices which might better characterize the atherogenic and thrombogenic potential of the diet than the simple approaches such as the P / S ratio. The atherogenic index (AI) and thrombogenic index (TI) take into account the different effects that single fatty acid might have on human health and in particular on the probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation. The recommended values of the AI are below 0.5 (Ulbricht and Southgate, 1991) and it was favorably decreased in the breast of the older birds ($P < 0.01$). The thrombogenic index was also significantly decreased ($P < 0.001$), while the h / H ratio increased ($P < 0.01$) in the

breast at 18 weeks of age. No such changes in the indices were observed in the thighs of the birds.

4 Conclusions

The results of the study showed that in a conventional system under controlled conditions, the age at slaughter has greater importance than the line when assessing the potential of the birds to produce high-quality meat in regard to its fatty acid profile. While the effect of the line was limited to differences in some individual fatty acids and related indices of lipid metabolism mainly in breast, age induced significant changes in the fatty acid composition in both muscles. Despite the decrease in C18:1 and MUFA, the proportion of C20:4n-6, C22:5n-3 and C22:6n-3 and the total PUFA increased in breast and thigh in the older birds. The positive effect of the later age on the fatty acid composition was associated with a decrease in the atherogenic and thrombogenic index, while h/H and P/S ratios increased significantly in breast. Additionally, in both muscles, the n-6/n-3 ratio decreased substantially in the older chickens. These results open new perspectives for further research on the breeding practices of La Belle and White Plymouth Rock lines in order to produce slow-growing chickens with a beneficial fatty acid composition of meat for the consumers.

Edited by: S. Maak

Reviewed by: A. Dalle Zotte and one anonymous referee

References

- Albert, C. M., Campos, H., Stampfer, M. J., Ridker, P. M., Manson, J. E., Willett, W. C., and Ma, J.: Blood levels of long-chain n-3 fatty acids and the risk of sudden death, *N. Engl. J. Med.*, 346, 1113–1118, doi:10.1056/NEJMoa012918, 2002.
- AOAC: Official methods of analysis, 18th Ed., Association of Official Analytical Chemists, Arlington, VA, USA, 2004.
- Bellizzi, M. C., Franklin, M. F., Duthie, G. G., and James, W. P. T.: Vitamin E and coronary heart disease: The European paradox, *Eur. J. Clin. Nutr.*, 48, 822–831, 1994.
- Bligh, E. G. and Dyer, W. Y.: A rapid method of total lipid extraction and purification, *Can. J. Biochem. Physiol.*, 37, 911–917, 1959.
- Calder, P.: The relationship between the fatty acid composition of immune cells and their function, *Prostaglandins Leukot. Essent. Fatty Acids*, 79, 101–108, doi:10.1016/j.biochi.2009.01.008, 2008.
- Choi, Y. J., Prak, Y. H., Michael, W. P., and Ntambi, J. M.: Regulation of stearoyl-CoA desaturase activity by the trans-10, cis-12 isomer of conjugated linoleic acid in HepG2 cells, *Biochem. Biophys. Res. Commun.*, 284, 689–693, doi:10.1006/bbrc.2001.5036, 2001.
- Christie, W. W.: Lipid analysis, Pergamon Press, Oxford, 1973.
- Dal Bosco, A., Mugnai, C., and Castellini, C.: Performance and meat quality of pure Ancona and Cornish × Ancona chickens organically reared, *Arch. Geflügelk.*, 75, 7–12, 2011.
- Dal Bosco, A., Mugnai, C., Ruggeri, S., Mattioli, S., and Castellini, C.: Fatty acid composition of meat and estimated indices of lipid metabolism in different poultry genotypes reared under organic system, *Poultry Sci.*, 91, 2039–2045, doi:10.3382/ps.2012-02228, 2012.
- Dal Bosco, A., Mattioli, S., Ruggeri, S., Mugnai, C., and Castellini, C.: Effect of slaughtering age in different commercial chicken genotypes reared according to the organic system: 2. fatty acid and oxidative status of meat, *It. J. Anim. Sci.*, 13, 462–466, doi:10.4081/ijas.2014.3311, 2014a.
- Dal Bosco, A., Mugnai, C., Roscini V., Paci G., and Castellini C.: Effect of genotype on estimated indexes of fatty acid metabolism in rabbits, *World Rabbit Sci.*, 22, 21–28, doi:10.4995/wrs.2014.1465, 2014b.
- De Smet, S., Raes, K., and Demeyer, D.: Meat fatty acid composition as affected by fatness and genetic factors, a review, *Anim. Res.*, 53, 81–98, 2004.
- Díaz, O., Rodríguez, L., Torres, A., and Cobos, A.: Fatty acid composition of the meat from Mos breed and commercial strain capons slaughtered at different ages, *Gracas y aceites*, 63, 296–302, doi:10.3989/gya.011312, 2012.
- Enser, M., Scollan, N., Gulati, S., Richardson, I., Nute, G., and Wood, J.: The effect of ruminally-protected dietary lipid on the composition and quality of beef muscle, in: *Proceedings of 47th International Congress of Meat Science and Technology*, Krakow, Poland, 26–31 August 2001, 186–187, 2001.
- Fanatico, A. C. and Born, H. M.: Label Rouge: Pasture-raised poultry in France, ATTRA publication, Natl. Center Appropriate Technol., Fayetteville, AR, 12 pp., 2001.
- Fanatico, A. C., Cavitt, L. C., Pillai, P. B., Emmert, J. L., and Owens, C. M.: Evaluation of slower-growing broiler genotypes grown with and without outdoor access: meat quality, *Poult. Sci.*, 84, 1785–1790, doi:10.1093/ps/84.11.1785, 2005.
- Fanatico, A. C., Pillai, P. B., Cavitt, L. C., Emmert, J. L., Meullenet, J. F., and Owens, C. M.: Evaluation of slower-growing broiler genotypes grown with and without outdoor access: sensory attributes, *Poult. Sci.*, 85, 337–343, doi:10.1093/ps/85.2.337, 2006.
- Fanatico, A. C., Pillai, P. B., Emmert, J. L., and Owens, C. M.: Meat quality of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access, *Poult. Sci.*, 86, 2245–2255, doi:10.1093/ps/86.10.2245, 2007.
- Fernandez, M. L. and West, K. L.: Mechanisms by which dietary fatty acids modulate plasma lipids, *J. Nutr.*, 135, 2075–2078, 2005.
- Ferrucci, L., Cherubini, A., Bandinelli, S., Bartali, B., Corsi, A., Lauretani, F., Martin, A., Andres-Lacueva, C., Senin, U., and Guralnik, J. M.: Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers, *J. Clin. Endocrinol. Metab.*, 91, 439–446, doi:10.1210/jc.2005-1303, 2006.
- Fetterman, J. and Zdanowicz, M.: Therapeutic potential of n-3 polyunsaturated fatty acids in disease, *Am. J. Health Syst. Pharm.*, 66, 1169–1179, doi:10.2146/ajhp080411, 2009.
- Figueras, M., Olivan, M., Busquets, S., Lopez-Soriano, F., and Argiles, J.: Effects of eicosapentaenoic acid (EPA) treatment on insulin sensitivity in an animal model of diabetes: Improvement of the inflammatory status, *Obesity*, 19, 362–369, doi:10.1038/oby.2010.194, 2011.

- Harris, W. S.: Omega-3 fatty acids and cardiovascular disease: A case for omega-3 index as a new risk factor, *Pharmacol. Res.*, 55, 217–223, doi:10.1016/j.phrs.2007.01.013, 2007.
- Høstmark, A. T. and Haug, A.: The inverse association between relative abundancies of oleic and arachidonic acid is relate of alfa-linolenic acid, *Lipids Health Dis.*, 13, 76, doi:10.1186/1476-511X-13-76, 2014.
- Kazala, E. C., Lozeman, F. J., Mir, P. S., Laroche, A., Bailey, D. R. C., and Weselake, R. J.: Relationship of fatty acid composition to intramuscular fat content in beef from crossbred Wagyu cattle, *J. Anim. Sci.*, 77, 1717–1725, doi:1999.7771717x, 1999.
- Komprda, T., Zelenka, J., Tieffová, P., Štohandlová, M., Foltýn, J., and Fajmonová, E.: Effect of age on total lipid, cholesterol and fatty acids content in tissues of fast and slow growing chickens, *Arch. Geflügelk.*, 64, 121–128, 2000.
- Kris-Etherton, P. M.: Monounsaturated fatty acids and risk of cardiovascular disease, *Circulation*, 100, 1253–1258, doi:10.1161/01.CIR.100.11.1253, 1999.
- Kris-Etherton, P. M., Griel, A. E., Psota, T. L., Gebauer, S. K., Zhang, J., and Etherton, T. D.: Dietary stearic acid and risk of cardiovascular disease: intake, sources, digestion, and absorption, *Lipids*, 40, 1193–1200, 2005.
- Lee, K. N., Pariza, M. W., and Ntambi, J. M.: Differential expression of hepatic stearyl-CoA desaturase gene 1 in male and female mice, *Biochim. Biophys. Acta.*, 1304, 85–88, doi:10.1016/S0005-2760(96)00145-2, 1996.
- Lu, J., Borthwick, F., Hassanali, Z., Wang, Y., Mangat, R., Ruth, M., Shi, D., Jaeschke, A., and Russell, J.: Chronic dietary n-3 PUFA intervention improves dyslipidaemia and subsequent cardiovascular complications in the JCR:LA-cp rat model of the metabolic syndrome, *Br. J. Nutr.*, 105, 1572–1582, doi:10.1017/S0007114510005453, 2011.
- Martino, G., Mugnai, C., Compagnone, D., Grotta, L., Del Carlo, M., and Sarti, F.: comparison of performance, meat lipids and oxidative status of pigs from commercial breed and organic cross-breed, *Animals*, 4, 348–360, doi:10.3390/ani4020348, 2014.
- Mensink, R. P. and Katan, M. B.: Effect of dietary fatty acids on serum lipids and lipoproteins, A meta-analysis of 27 trials, *Arterioscler. Thromb.*, 12, 911–919, doi:10.1161/01.ATV.12.8.911, 1992.
- Mensink, R. P.: Effects of stearic acid on plasma lipid and lipoproteins in humans, *Lipids*, 40, 1201–1205, 2005.
- Michalczyk, M., Józwiak, A., Damaziak, K., Zdanowska-Sąsiadek, Z., Marzec, A., Gozdowski, D., and Strzałkowska, N.: Age-related changes in the growth performance, meat quality, and oxidative processes in breast muscles of three chicken genotypes, *Turk. J. Vet. Anim. Sci.*, doi:10.3906/vet-1502-64, 2016.
- Ntambi, J. M.: Regulation of stearyl-CoA desaturase by polyunsaturated fatty acids and cholesterol, *J. Lipid Res.*, 40, 1549–1558, 1999.
- Petkov, E.: Comparative study of three type combinations chickens with different growth rate, PhD, Institute of Animal Science-Kostinbrod, Agricultural Academy, Sofia, 2015.
- Petkov, E., Popova, T., Todorova, M., and Ignatova, M.: Quality traits of breast and thigh muscles in three chicken genotypes, *World's Poultry Sci. J.*, 69, Suppl., 5, 2013.
- Poltowicz, K. and Doktor, J.: Effect of slaughter age on performance and meat quality of slow-growing broiler chickens, *Ann. Anim. Sci.*, 12, 621–631, doi:10.2478/v10220-012-0052-0, 2012.
- Popova, T., Petkov, E., and Ignatova, M.: Meat quality of La Belle and White Plymouth Rock chickens slaughtered at different age, *Agric. Sci. Technol.*, 8, 162–165, doi:10.15547/ast.2016.02.030, 2016.
- Popova, T., Ignatova, M., and Petkov, E.: Lipid composition of meat in male layer-type chickens at different age, in: *Proceedings of the 4th International congress New Perspectives and Challenges in Livestock Production*, Belgrade, 7–9 October, 2015, 168–175, 2015.
- Poureslami, R., Turchini, G. M., Raes, K., Huyghebaert, G., and De Smet, S.: Effect of diet, sex and age on fatty acid metabolism in broiler chickens: SFA and MUFA, *Br. J. Nutr.*, 104, 204–213, doi:10.1017/S0007114510000541, 2010.
- Raes, K., De Smet, S., and Demeyer, D.: Effect of double-muscling in Belgian Blue young bulls on the intramuscular composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids, *Anim. Sci.*, 73, 253–260, 2001.
- Raitt, M. H., Connor, W. E., Morris, C., Kron, J., Halperin, B., Chugh, S. S., McClelland, J., Cook, J., and MacMurdy, K.: Fish oil supplementation and risk of ventricular tachycardia and ventricular fibrillation in patients with implantable defibrillators: A randomized controlled trial, *JAMA-J Am. Med. Assoc.*, 293, 2884–2891, doi:10.1001/jama.293.23.2884, 2005.
- Rondanelli, M., Giacosa, A., Opizzi, A., Pelucchi, C., La Vecchia, C., Montorfano, G., Negroni, M., Berra, B., Politi, P., and Rizzo, A.: Long chain omega 3 polyunsaturated fatty acids supplementation in the treatment of elderly depression: Effects on depressive symptoms, on phospholipids fatty acids profile and on health-related quality of life, *J. Nutr. Health Aging* 15, 37–44, doi:10.1007/s12603-011-0011-y, 2011.
- Santos-Silva, J., Bessa, R. J. B., and Santos-Silva, F.: Effect of genotype, feeding system and slaughter weight on the quality of light lambs – II. Fatty acid composition of meat, *Livest. Prod. Sci.*, 77, 187–192, doi:10.1016/S0301-6226(02)00059-3, 2002.
- Schmitz, G. and Ecker, J.: The opposing effects of n-3 and n-6 fatty acids, *Prog. Lipid Res.*, 47, 147–155, doi:10.1016/j.plipres.2007.12.004, 2008.
- Siriwardhana, N., Nishan, S., Kalupahana, S., and Naima Moustaid-Moussa, N.: Chapter 13- Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid, *Advances in Food and Nutritional Research*, 65, 211–222, doi:10.1016/B978-0-12-416003-3.00013-5, 2012.
- Ulbricht, T. L. and Southgate, D. A. T.: Coronary heart disease: seven dietary factors, *Lancet*, 338, 985–992, doi:10.1016/0140-6736(91)91846-M, 1991.
- Westgren, R. E.: Delivering food safety, food quality, and sustainable production practices: The Label Rouge poultry system in France, *Am. J. Agric. Econ.*, 81, 1107–1111, doi:10.2307/1244092, 1999.
- Wood, J. V., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., and Whittington, F. M.: Fat deposition, fatty acid composition and meat quality: A review, *Meat Sci.*, 78, 343–358, doi:10.1016/j.meatsci.2007.07.019, 2008.
- Wortman, P., Miyazaki, Y., Kalupahana, N. S., Kim, S., Hansen-Petrik, M., Saxton, A. M., Claycombe, K. J., Voy, B. H., Whelan, J., and Moustaid-Moussa, N.: n3 and n6 polyunsaturated fatty acids differentially modulate prostaglandin E secretion but not markers of lipogenesis in adipocytes, *Nutr. Metab.*, 6, 5, doi:10.1186/1743-7075-6-5, 2009.

Yu, S., Derr, J., Etherton, T. D., and Kris-Etherton, P. M.: Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic, *Am. J. Clin. Nutr.*, 61, 1129–1139, 1995.

Zhang, S., Knight, T. J., Stalder, K. J., Goodwin, R. N., Lonergan, S. M., and Beitz, D. C.: Effects of breed, sex, and halothane genotype on fatty acid composition of pork longissimus muscle, *J. Anim. Sci.*, 85, 583–591, doi:10.2527/jas.2006-239, 2007.