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Original article

Fatty Acid Composition of Thigh Meat in Two Lines of Slow-Growing Chickens as Affected by the Access to Pasture

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Abstract

The study was carried out to examine the effect of the pasture access vs. conventional rearing on the fatty acid composition an of the thigh meat in two lines of slow growing chickens - La Belle (LB) and Bresse Gauloise (BB). Additionally differences between the lines were also examined. The influence of both factors on the lipid profile were assessed through two-way ANOVA. The effect of pasture was more pronounced than the line and was associated with lower contents of the saturated (SFA) (P<0.001), and significant increase (P<0.001) of the polyunsaturated fatty acids (PUFA). Such changes in the fatty acids of the thigh meat in the chickens having access to pasture induced considerably lower atherogenic (AI) and thrombogenic (TI) indices. Furthermore, the n-6/n-3 ratio was reduced (P<0.001), while the ratios between the poly- and saturated fatty acids (P/S), as well as the hypo- and hypercholesterolemic (h/H) (P<0.001) were increased in the pastured lines. Differences in the fatty acid composition of the thigh meat due to the line of the birds, were not observed, however the BB birds reared conventionally tended to have higher content of C18:2n-6 and C18:3n-3.

Keywords: Fatty acids, Meat, Pasture access, Slow-growing chickens.

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INTRODUCTION

Chicken meat, especially breast, is characterised with high nutritional value and dietetic value, due to its high protein and low fat content. At the same time, it has high levels of essential fatty acids and fat soluble vitamins. While some consumers may switch from white to dark meat (thighs) because of a taste preference, it is known that this kind of chicken meat has higher lipid content (Sirri et al., 2011; Chen et al., 2013; Popova et al., 2016) – a parameter that is closely related to the nutritional and healthy value of the meat and food as a whole. The fatty acid composition of the meat lipids can be modified through various strategies for poultry rearing. Access to pasture has proven to be effective for favourable manipulation of the fatty acid profile in farm animals, mainly by increasing the content of the polyunsaturated n-3 fatty acids (Bee et al., 2004; Lebret and Guillard, 2005; Popova, 2007). Autochthonous chicken lines are slower growing and are well adapted to outdoors rearing with pasture access. In recent years, such lines have been gaining much popularity due to their higher resistance compared to commercial broilers and also their unique meat quality characteristics. The slower growing lines involved in this study are the Bulgarian La Belle –and Bresse Galouise - known to be associated with production of high quality poultry products in France. Studies on both lines have been relatively scarce, concerning their carcass traits and meat chemical composition (Popova et al., 2017) when the birds were reared in conventional system. Recently the effect of the pasture access on the fatty acid profile of the breast meat in these lines has also been investigated (Popova et al., 2018). In the present study male chickens from La Belle and Bresse Gauloise were reared conventionally and outdoors with pasture access and the fatty acid profile and lipid nutritional indices have been determined in the thigh meat.

Material and methods

Experimental birds and rearing systems

Two trials were carried out respectively in the experimental poultry farm of the Institute of Animal Science –Kostinbrod, Bulgaria (conventional rearing) and Livadi symbiotic farm located in Damyanitsa village, Bulgaria (pasture rearing) with male slow-growing chickens of two lines La Belle (LB) and Bresse Gauloise (BB). For the first trial, a total of 73 LB and 51 BB 1-day old male chickens obtained from the parent stock in the Institute were placed into a deep litter facility with a stocking density of 14 birds/m^2 in separate pens in the same poultry house in the Institute. All the birds were fed *ad libitum* starter (ME- 13.18MJ/kg; protein content-19.41%) and finisher (ME -13.00 MJ/kg, protein content-17.77%) diets, respectively for 4 and 8 weeks. Water was provided *ad libitum* with a nipple drinker. The lighting regime was 15 h of light and 9 h of darkness, and the temperature ranged between 20 and 24 (started from 32-36°C in the first 3 days after hatching, followed by a programmed decrease). In the second trial, the total number of male chickens hatched and reared in Livadi farm was 48, divided into two groups, each containing 21 and 27 chickens according to the line – LB and BB. For a period of 3 weeks after hatching, the chickens were kept in controlled microclimate conditions (as described by Salatin, 1998). From 4 to 12 weeks of age, the birds were reared outdoors in wooden cages covered inside with aluminium plates to prevent the overheating of the chickens. The cages were equipped with nipple drinkers and feeders while being open so that the birds could access pasture. Additionally, the chickens were fed *ad libitum* the same diet as the ones reared conventionally in the first trial. The fatty acid composition of the diets and grass is presented in Table 1.

Table 1. Fatty acid composition (% FAME) of the diet and grass

Fatty acid	Starter (1-28d)	Finisher (29 d +)	Grass (29 d +)
C14:0	0.19	0.09	1.48
C16:0	16.38	14.30	18.10
C16:1n-7	0.26	0.22	2.98
C18:0	2.75	2.63	2.82
C18:1n-9	25.97	28.76	5.85
C18:2n-6	53.43	52.34	20.35
C18:3n-3	1.01	1.65	48.41

Slaughtering and sampling

At 12 weeks of age, 6 chickens of each line from both trials (rearing systems) were selected for slaughter based on the average live weight (LB conventional-1986.67±35.03g; BB conventional - 1973.83±37.61g; LB pasture-1317.67±67.04g; BB pasture-1370.66±60.71g). 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 thigh muscles of each chicken carcass were separated, minced with a meat grinder, and samples for the determination of the fatty acid profile (10 g) were taken, vacuum-packed and stored at −20◦C until analysis.

Fatty acid analysis

Total lipids from the thigh meat 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×0.25 mm×0.20 μm) 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◦C/min and then held for 5 min. The temperatures of the detector and injector were 230◦C. Methyl esters were identified through 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).

Calculations and statistical evaluation

To evaluate the activity of both Δ5-desaturase and Δ6-desaturase, the enzymes catalyzing the formation of long-chain n-6 and n-3 PUFA from their respective precursors C18:2n-6 and C18:3n-3, the following equation as proposed by Sirri et al. (2010):

Δ5-desaturase + Δ6-desaturase = [C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3/C18:2n-6 +C18:3n-3 + C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3] × 100.

Furthermore, the amount of each fatty acid was used to calculate the indices of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht and Southgate (1991):

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

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

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

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

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

Results

Fatty acid composition

The rearing system influenced substantially the fatty acid profile of the thigh meat in the chickens (Table 2).

Table 2. Fatty acid composition (% FAME) in the thigh meat in La Belle (LB) and Bresse Gauloise (BB) male chickens reared conventionally or with pasture access (values least squares means)

Fatty acid	Conventional		Pasture access		S.E.[1]	Rearing system	Line	Rearing system x Line
	LB	BB	LB	BB				
C14:0	1.04	1.03	0.88	0.90	0.20	0.09	NS	NS
C16:0	30.99	30.70	24.66	24.58	2.92	***	NS	NS
C16:1n-9	0.31	0.29	0.34	0.33	0.07	NS	NS	NS
C16:1n-7	8.65	7.96	7.62	7.18	1.54	NS	NS	NS
C18:0	7.57	7.54	7.36	6.70	0.75	NS	NS	NS
C18:1n-9	35.55	32.59	32.95	34.87	3.21	NS	NS	NS
C18:2n-6	14.98	18.03	21.20	21.67	2.21	***	0.06	NS
C18:3n-3	0.30	0.45	0.65	0.67	0.10	***	0.06	NS
C20:2n-6	0.13	0.17	0.22	0.19	0.06	NS	NS	NS
C20:3n-6	0.09	0.12	0.25	0.23	0.08	***	NS	NS
C20:4n-6	0.36	1.03	3.24	2.23	0.81	***	NS	*
C20:5n-3	0.00	0.00	0.02	0.01	0.02	0.08	NS	NS
C22:5n-3	0.03	0.06	0.34	0.27	0.06	***	NS	0.07
C22:6n-3	0.00	0.03	0.27	0.17	0.05	***	NS	**

[1] S.E. standard error; * P<0.05; **P<0.01; ***P<0.001

Both lines reared outdoors had significantly lower content of C16:0 (P<0.001) as well as tending to decrease C14:0 (P<0.09). Access to pasture resulted in considerably higher levels of C18:2n-6 (P<0.001), C18:3n-3 (P<0.001) and C20:3n-6 (P<0.001) in LB and BB birds. Furthermore, the contents of C20:4n-6, C22:5n-3 and C22:6n-3 showed dramatic increase in the pastured birds. Although not significantly different the content of C20:5n-3 also tended to increase as a result of the outdoors rearing.

Significant differences in the fatty acid profile between the lines were not detected, although BB reared conventionally showed a tendency toward higher contents of C18:2n-6 and C18:3n-3. Both rearing conditions and line of the birds interacted significantly in regard to the contents of C20:4n-6 (P<0.05) and C22:6n-3 (P<0.01).

The content of the total saturated fatty acids (Table 3) followed the pattern of C16:0 and was significantly decreased in the birds on pasture (P<0.001), while the total PUFA and n-6 were increased (P<0.001).

Table 3. Total amount of fatty acids in the thigh meat in La Belle (LB) and Bresse Gauloise (BB) male chickens reared conventionally or with pasture access (values least squares means)

Item	Conventional		Pasture access		S.E.[1]	Rearing system	Line	Rearing system x Line
	LB	BB	LB	BB				
SFA	39.60	39.27	32.90	32.18	2.68	***	NS	NS
MUFA	44.51	40.84	40.91	42.38	2.82	NS	NS	NS
PUFA	15.89	19.89	26.19	25.44	2.76	***	NS	NS
Σn-6	15.56	19.35	24.91	24.32	2.66	***	NS	NS
Σn-3	0.33	0.54	1.28	1.12	0.10	***	NS	*
Δ5+Δ6	3.29	6.52	15.77	11.38	3.68	***	NS	*

[1] S.E. standard error; * P<0.05; ***P<0.001

The access to pasture induced more than a double increase in the total n-3 PUFA as well (P<0.001), however this factor significantly interacted with the line of the chickens (P<0.05). The increase in the content of the long chain PUFA in the pastured birds was additionally confirmed by the values of the Δ5+Δ6 desaturase index, which were significantly augmented in the lines reared outdoors (P<0.001).

Lipid nutritional indices

The access to pasture affected all the nutritional indices presented in Table 4.

Table 4. Lipid nutritional indices of the thigh meat in La Belle (LB) and Bresse Gauloise (BB) reared conventionally or with pasture access (values least squares means)

Item	Conventional		Pasture access		S.E.[1]	Rearing system	Line	Rearing system x Line
	LB	BB	LB	BB				
P/S	0.40	0.51	0.80	0.79	0.09	***	NS	NS
n-6/n-3	47.15	35.83	19.46	21.71	5.34	***	NS	*
AI	0.58	0.57	0.42	0.41	0.08	***	NS	NS
TI	1.28	1.23	0.89	0.88	0.14	***	NS	NS
h/H	1.61	1.65	2.31	2.37	0.31	***	NS	NS

[1] S.E. standard error; * P<0.05; ***P<0.001

The ratios P/S and h/H were considerably augmented, while n-6/n-3 were twice reduced in the pastured birds (P<0.001). However, significant interaction with the line was observed in regard to the values of n-6/n-3. Furthermore the values of AI were lower in the birds having access to pasture (P<0.001). On the other hand, this was not observed for the values of TI, where significant interaction of the factors was found. The BB birds reared indoors had markedly higher TI when compared to the LB, however the values of the index did not differ between the pastured lines.

Discussion

The fatty acid composition of meat depends largely on the rearing conditions of the farm animals (Popova, 2007, 2014; Lebret, 2008) and the poultry is no exception. In this study, the access to pasture influenced positively the fatty acid profile of thigh meat, mainly in terms of the saturated and polyunsaturated fatty acids. As mentioned above, chickens reared outdoors displayed lower content of SFA, associated with the decrease in C16:0. Our results are in line with those of Michalczuk et al. (2017) who observed marked decrease in the total SFA in slow-growing lines, reared outdoors. On the other hand, Cömert et al. (2016) found increase in the contents of C16:0 in slow-growing chickens, reared organically with outdoor access which is contrary to the results we had. However, Molee et al. (2012) and Sosnówka-Czajka et al. (2017) did not observe any effect of the rearing system (pasture vs. conventional) on the saturated fatty acids. Although the saturated fatty acids have very important roles in the organism in gene transcription, lipogenesis and regulation of PUFA bioavailability (Legrand and Rioux, 2010), still it has been shown that the reduction of their levels (particularly C14:0 and C16:0), or replacement by polyunsaturated fatty acids, diminishes the risk of the cardiovascular diseases (Hooper et al., 2015; Zong et al, 2016, Biggs et all., 2017, Nettleton et al., 2017). Hence, the reduction of the SFA content and C16:0 might be considered positive for the healthy value of the chicken meat.

The results of our study show that the thigh meat of pastured bird displayed increase in C18:2n-6 leading to augmentation of C20:4n-6 and C18:3n-3, associated mostly with higher levels of C22:5n-3 and C22:6n-3. Consequently the total amounts of n-6 and n-3 PUFA in the meat of birds on pasture were higher when compared to the conventionally reared chickens. In line with our results, Sosnówka-Czajka et al. (2017) reported increase in both n-6 and n-3 in the slow growing lines reared organically. Higher contents of n-3 PUFA in meat due to pasture rearing was found also by Castellini et al. (2002) and Husak et al. (2008), while Molee et al. (2012) observed decrease in n-6 PUFA and no change in n-3 PUFA in the thigh meat of birds reared outdoors. Usually, rearing on pasture induces dramatic increase in C18:3 of the meat in farm animals, due to the high content of this fatty acid in the grass, while on the other hand, concentrate and grains are richer in C18:2n-6 (Table 1). It could be suggested that the augmentation of C18:2n-6 in the chickens reared on pasture is due to the addition of concentrate in the diet. It is known that both C18:2n-6 and C18:3n-3 are essential and could not be synthesised by the animals through endogenous synthesis but derived exclusively from the diet. However, in the organism they can be converted to a polyunsaturated fatty acids with a longer carbon chain through elongation and desaturation, catalysed by delta-6 and delta-5 desaturases. These enzymes are rate limiting in the synthesis of C20:4n-6, C20:5n-3 and C22:6n-3 for the respective precursors- C18:2n-6 and C18:3n-3 (Cho et al., 1999, Dunbar and Bauer, 2002). The results of the present study showed considerably higher $\Delta5+\Delta6$ desaturase activity in the pastured birds. As mentioned above the content C18:3n-3 in the grass is much higher than that of the feed (48.41%). Rymer and Givens (2005) reported that the increased levels of C18:3n-3 in the diet of the birds can result in sufficiently increased content of this fatty acid in the tissues which is not noticeable for C20:5n-3 and C22:6n-3. These authors also reported that desaturase activity can be influenced by the dietary factors but also depends on the genetic factors which is confirmed by our results, showing the significant interaction between the two factors of interest in this study.

Although the rearing system and the line of the birds interacted significantly in regard to the n-6/n-3 ratio, it was almost twice lower in the pastured birds, with values within the range of 19.46- 21.71. Such values are considerably higher than the limit of 4 (Simopoulos, 2004) and it could be suggested that pasture alone is not sufficient to reduce the n-6/n-3 ratio. The observed high values of n-6/n-3 might be successfully compensated by the positively augmented values of P/S, higher than 0.4 (Wood et al., 2003) and the ratio h/H. In order to characterise the dietetic quality of the thigh meat in the slow-growing chickens as affected to the rearing system, the atherogenic and thrombogenic indices were calculated. The values of both were strongly influenced by the rearing system, showing lower values in the two lines having access to pasture. Thrombogenic index in the thigh meat of the pastured chickens varied 0.88-0.89. In our previous study on the breast meat, values of TI in the pastured birds were 0.90-1.02. The AI was within the range of 0.41-0.42 in the thigh meat of the pastured birds, while in the birds reared conventionally the values varied between 0.57-0.58. According to Ulbricht and Southgate (1991), the values of AI should not be higher than 0.5, while according to Knock (2007) AI <1 is desirable for human health. In both cases the results of our study show that poultry meat, particularly from the chicks reared on pasture has good dietetic value.

Conclusions

The access to pasture affected to a greater extent than the line the fatty acid composition of the thigh meat. In general, its influence was positive and associated with lower contents of hypercholesterolemic C16:0 and total SFA. On the other hand, significant increase was observed in the pastured birds in terms of the PUFA, which led to significant improvement of the dietetic quality of the thigh meat displaying lower atherogenic index n-6/n-3 ratio, while higher values of P/S and h/H indices.The results of our work appear promising and reveal potential for further research concerning the rearing practices of the indigenous slow-growing lines in order to produce high quality meat with a healthier lipid profile.

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