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Research Article

Fatty acid composition of breast meat in two lines of slow-growing chickens reared conventionally or on pasture

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Abstract

The study was carried out to compare the fatty acid composition of the breast meat in two lines of slow growing chickens - La Belle (LB) and Bresse Gauloise (BB), reared conventionally or having access to pasture. The differences in the lipid profile due to the lines and rearing strategies were assessed through two-way ANOVA. Both factors influenced the lipid composition in the breast meat, however the effect of pasture was more pronounced. It was associated with lower contents of C14:0, C16:0 and C16:1Δ7 ($P<0.001$), C18:1 ($P<0.05$), total saturated (SFA) and monounsaturated fatty acids (MUFA) ($P<0.01$), and significant increase ($P<0.001$) of the long chain polyunsaturated fatty acids (PUFA). These changes in the lipid profile of the breast meat in the chickens reared outdoors led to considerably lower atherogenic and thrombogenic indices, reduced ω -6/ ω -3 ratio ($P<0.001$), as well as higher P/S and h/H values ($P<0.001$). Differences in the fatty acid composition of the breast meat were also due to the line of the birds, showing advantage of the LB over BB chickens. The former had lower contents of saturated ($P<0.001$) but higher long chain polyunsaturated fatty acids ($P<0.05$) which determined better nutritional indices of the breast meat in the LB line.

Keywords: slow growing chickens, fatty acid composition, meat, indoors, pasture

Abbreviations

AI	– atherogenicity index	MUFA	– monounsaturated fatty acids
ANOVA	– analysis of variance	PUFA	– polyunsaturated fatty acids
BB	– Bresse Gauloise	SFA	– saturated fatty acids
GLC	– gas-liquid chromatography	TI	– thrombogenicity index
FAME	– fatty acids methyl esters		
LB	– La Belle		

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Introduction

Today, a greater proportion of consumers are interested in poultry meat products derived from alternative production systems. In Europe, such systems use slower-growing lines slaughtered at least at 81 days of age that are fed on cereal-based diets, having outdoor access, however with a limited intake of grass biomass (Ponte et al. 2008). According to Fanatico et al. (2005, 2006), slow-growing chicken lines require a longer fattening period and are well adapted to outdoors rearing conditions. Indigenous chicken lines are also slower-growing and recently have been drawing much attention among consumers desiring higher quality and safety of poultry products.

Such lines have some advantages over the commercial broiler chickens in terms of sensory characteristics of meat and also they appear to be much more resistant to pathogens and environmental stressors (Nielsen et al. 2003; Sirri et al. 2010). La Belle line originates from Bulgaria, while the old French Bresse Gauloise has been only recently imported to our country. While Bresse Gauloise, principally known as meat breed, is associated with production of high quality poultry products in France, the information on La Belle meat quality traits remains relatively scarce (Popova 2016a,b). Both lines have slower growth and as shown by Baéza et al. (2009) and Popova et al. (2016a), at least 120 days are necessary for the birds to reach a live weight of 2.5 kg.

Rearing outdoors on pasture appears to be a successful strategy for increasing the contents of ω -3 PUFA in the meat of farm animals and to manipulate its lipids towards more favourable profile (Popova 2007). However, in poultry, the influence of the combined effect of the line and the access to pasture on the fatty acid profile of meat from such alternative production systems remains unknown. Hence, the aim of the study was to compare the fatty acid composition in the breast meat in chickens, as affected by the line and the rearing system.

Materials and Methods

Experimental birds and rearing systems. The experiment consisted of two trials carried out in the experimental poultry farm of the Institute of Animal Science - Kostinbrod (conventional rearing) and Livadi symbiotic farm located in Damyantsa village (pasture rearing) with male slow-growing chickens of the two lines La Belle (LB) and Bresse Gauloise (BB). 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.m² in separate pens in the same poultry house in the Institute. All the birds were fed *ad libitum* starter (ME- 13.18MJ.kg⁻¹; protein content- 19.41%) and finisher (ME -13.00 MJ.kg⁻¹, protein content - 17.77%), 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°C (started from 32-36°C in the first 3 days after hatching, followed by technologically programmed decrease). The total number of male chickens reared in Livadi farm was 48, divided into two groups, each containing 21 and 27 chickens according to the line – LB and BB. The birds were reared in controlled microclimate conditions until 3 weeks of age (as described by Salatin, 1998). From 4 to 12 weeks of age, the birds were reared 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 have access to pasture. Additionally, the chickens were fed *ad libitum* the same diet as the ones from the trial in the Institute. The fatty acid composition of the diets is presented in Table 1.

Slaughtering and sampling. At 12 weeks of age, 6 birds 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 breast muscles (m. *Pectoralis profundus et superficialis*) of each chicken were separated, 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.

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

Fatty acid	Starter 1-28 d	Finisher 29 d +	Grass 29 d +
C14:0	0.19	0.09	1.48
C16:0	16.38	14.30	18.10
C16:1Δ7	0.26	0.22	2.98
C18:0	2.75	2.63	2.82
C18:1	25.97	28.76	5.85
C18:2ω-6	53.43	52.34	20.35
C18:3ω-3	1.01	1.65	48.41

Fatty acid analysis. Total lipids from the skinless breast 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⁻¹ 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). 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):

$$AI=(4\times C14:0+C16:0)/[MUFA+\Sigma(\omega-6)+\Sigma(\omega-3)] \quad (1)$$

$$TI=(C14:0+C16:0+C18:0)/[0.5\times MUFA+0.5\times(\omega-6)+3\times(\omega-3)+(\omega-3)/(\omega-6)] \quad (2)$$

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

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

Statistical evaluation. The data were statistically evaluated by two-way ANOVA as the line of the birds, the rearing system 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).

Results and Discussion

The rearing system affected the contents of the individual fatty acids to a greater extent than the line of the birds (Table 2). The LB and BB chickens that had access to pasture had lower contents of C14:0 and C16:0 (P<0.001), while the levels of C18:0 were higher in these birds (P<0.05). On the other hand both C16:0 and C18:0 contents in the breast meat differed significantly between the lines, showing higher values in the BB birds compared to LB. The chickens that were reared with access to pasture displayed significantly lower amounts of C16:1Δ7 (P<0.001) and C18:1 (P<0.05) in the meat, however no differences between the lines were observed in regard to these fatty acids. The contents of the individual polyunsaturated fatty acids in the breast meat were influenced by the rearing conditions of the chickens. Considerable increase (P<0.001) was observed in the amounts of C20:3ω-6, C20:4ω-6, C20:5ω-3, C22:5ω-3 and C22:6ω-3 in the breast muscle of the birds on pasture. The last three fatty acids were affected by the line of the chickens as well, showing higher amounts in LB birds (P<0.05).

Similar to the changes of the contents of the individual fatty acids, the total amounts of SFA, MUFA, PUFA, ω-6 and ω-3 PUFA in the breast of the slow-growing lines were affected mostly by the rearing system (Table 3). Both total SFA and

Table 2. Fatty acid composition (% of total FAME) in the breast meat of La Belle (LB) and Bresse Gauloise (BB) chickens according to the line and rearing system (values least squares means)

Fatty acid	La Belle (LB)		Bresse Gauloise (BB)		S.E.	Significance of the factors		
	Conventional	Pasture access	Conventional	Pasture access		Line	Rearing system	Line × Rearing system
C14:0	0.68	0.54	0.71	0.51	0.07	NS	***	NS
C16:0	27.58	24.49	28.68	26.27	0.96	***	***	NS
C16:1Δ9	0.13	0.12	0.11	0.12	0.02	NS	NS	NS
C16:1Δ7	5.79	4.43	5.54	4.16	0.86	NS	***	NS
C18:0	7.78	8.75	8.71	9.38	0.81	*	*	NS
C18:1	34.60	31.96	34.76	32.45	2.53	NS	*	NS
C18:2ω-6	19.96	20.72	19.53	19.70	1.84	NS	NS	NS
C18:3ω-3	0.48	0.49	0.47	0.45	0.07	NS	NS	NS
C20:2ω-6	0.25	0.27	0.23	0.23	0.07	NS	NS	NS
C20:3ω-6	0.28	0.52	0.22	0.55	0.11	NS	***	NS
C20:4ω-6	2.18	6.43	0.95	5.31	1.44	NS	***	NS
C20:5ω-3+C22:5ω-3+ C22:6ω-3	0.29	1.28	0.09	0.87	0.28	*	***	NS

S.E. – standard error; *P<0.05; **P<0.01;***P<0.00

Table 3. Total amounts of saturated, mono - and polyunsaturated fatty acids in the breast meat of La Belle (LB) and Bresse Gauloise (BB) chickens according to the line and rearing system (values least squares means)

Fatty acid	La Belle (LB)		Bresse Gauloise (BB)		S.E.	Significance of the factors		
	Conventional	Pasture access	Conventional	Pasture access		Line	Rearing system	Line × Rearing system
SFA	36.04	33.78	38.10	36.16	1.34	***	**	NS
MUFA	40.52	36.51	40.41	36.73	3.17	NS	**	NS
PUFA	23.44	29.71	21.49	27.11	2.46	*	***	NS
ω-3	0.77	1.77	0.56	1.32	0.28	*	***	NS
ω-6	22.67	27.94	20.93	25.79	2.32	NS	***	NS

S.E. – standard error; *P<0.05; **P<0.01;***P<0.001

Table 4. Lipid nutritional indices in the breast meat of La Belle (LB) and Bresse Gauloise (BB) chickens according to the line and rearing system (values least squares means)

Fatty acid	La Belle (LB)		Bresse Gauloise (BB)		S.E.	Significance of the factors		
	Conventional	Pasture access	Conventional	Pasture access		Line	Rearing system	Line× Rearing system
ω-6/ω-3	29.44	15.79	37.38	19.54	4.16	**	***	NS
P/S	0.65	0.88	0.56	0.75	0.06	***	***	NS
AI	0.47	0.40	0.51	0.44	0.02	**	***	NS
TI	1.06	0.90	1.18	1.02	0.05	***	***	NS
h/H	2.05	2.48	1.91	2.22	0.12	**	***	NS

S.E. – standard error; **P<0.01;***P<0.001

MUFA were lower in the chickens on pasture ($P<0.01$), while the opposite was observed in regard to PUFA ($P<0.001$), and these changes corresponded to the above mentioned concerning the individual fatty acids. In addition to the effect of the rearing system, significant differences in the total amounts of the fatty acids in breast meat existed between the lines as well. The total SFA was higher in the BB line ($P<0.001$), while these chickens displayed lower content of PUFA ($P<0.05$).

The access to pasture of the chickens from both slow-growing lines positively influenced the nutritional indices determined for the breast muscle in this study (Table 4). In general, the values of the ω -6/ ω -3 PUFA, AI and TI decreased substantially while P/S and h/H were increased in the chickens on pasture ($P<0.001$). The line of the birds also induced significant differences in the nutritional indices in the breast, showing advantage for the LB chickens. They had higher values of P/S ($P<0.001$) and h/H ($P<0.01$) ratios, while lower ω -6/ ω -3 ($P<0.01$), AI ($P<0.01$) and TI ($P<0.001$) values when compared to BB line. The results of the study demonstrated the advantage of the pasture rearing of the slow-growing chickens compared to the indoors for favourable manipulation of the fatty acid composition of the meat.

The lipids of the breast meat in the chickens having access to pasture had lower saturation and contents of the hypercholesterolaemic C14:0 and C16:0. On the other hand, the monounsaturated fatty acids were also decreased in the pastured birds. This could be associated to the differences in the total lipid content of the breast meat due to the rearing system in the chickens from both lines (1.59% vs. 0.96% for the conventionally and pastured reared), since it is known that the content of MUFA and especially C18:1 correlates with the intramuscular lipid content (Smith et al. 2009). Similar to us, Michalczuk et al. (2017) reported lower levels of SFA in the breast meat of slow-growing chickens reared outdoors on pasture when compared to the indoors system, however they observed higher MUFA content in the pastured chickens. Cömert et al. (2016) observed increased content of SFA and MUFA in the drumstick of both fast and slow-growing chicken genotypes

when reared in organic system with pasture access compared to conventional system, and also surprisingly reported decreased content of the ω -6 and ω -3 PUFA in the chickens from the pastured groups. This contradicts to our results concerning the contents of SFA, MUFA and PUFA. Although it is known that grass from pastures contain substantially higher amounts of C18:3 ω -3, the percentage of this fatty acid remained unaffected in the breast meat of both lines that were reared outdoors. The same was observed in terms of C18:2 ω -6. These two fatty acids are essential and are exclusively derived from the diet. It could be suggested that the lack of effect of the pasture access is due to the presence of concentrate in the diet of the birds. On the other hand, the substantially increased levels of ω -3 and ω -6 PUFA indicated intensive conversion of the precursors (C18:3 ω -3 and C18:2 ω -6) in the pastured birds compared to the conventionally reared ones. In their work, Ponte et al. (2008) reported even reduced content of C18:3 ω -3 in pastured broilers, especially during the spring season and augmented levels of C22:2 ω -6 and C22:4 ω -6 depending however on the type of pasture.

The positive influence of the pasture access in the two lines of slow-growing chickens was associated with significant improvement in the ratio ω -6/ ω -3. It could be seen that it was approximately twice reduced in the pastured birds, however its values were high above the recommended (<4.0) according to Scollan et al. (2006). This indicated the relative imbalance of the chicken meat in regard to this trait but also demonstrated the possibilities of the pasture rearing to successfully decrease its values. In both lines reared indoors and on pasture the values of the ratio P/S were above the recommended minimum of 0.4 (Wood et al. 2003), as in the breast of the chickens having access to pasture the ratio was beneficially increased.

The atherogenic and thrombogenic indices have been introduced by Ulbricht and Southgate (1991) in attempt to take into account the different roles of the different fatty acids for the human health. In this study, their values were lower in the birds reared on pasture. AI varied within the range of

0.47-0.51 and 0.40-0.44 for the chickens reared indoors and on pasture, while TI was 1.06-1.18 and 0.90-1.02, respectively. These values for both indices were close to the reported in our previous study (Popova et al. 2016b), and also to the ones obtained by Del Puerto et al. (2017). According to Ulbricht and Southgate (1991), the recommended values of AI should be below 0.5, and the ratio was positively affected by the rearing system in this study.

Although not very pronounced, differences between the lines in regard to some individual fatty acids as well as their total amounts and lipid nutritional indices were observed in this study. The chickens of LB lines showed better fatty acid profile when compared to BB. They had lower contents of SFA but higher amount of long chain PUFA which determined also better nutritional indices of the breast muscles in LB slow-growing line. So far, comparisons between indigenous chicken lines in terms of their meat quality and fatty acid composition are very scarce in Bulgaria. In a previous study we compared La Belle and White Plymouth Rock lines slaughtered at different age, however the differences due to the line were limited to only four fatty acids in breast and one in thigh meat (Popova et al. 2016b).

Conclusions

The access to pasture affected to a greater extent than the line the fatty acid composition of the breast meat. In general, its influence was positive and associated with lower contents of hypercholesterolemic C14:0 and C16:0, as well as C16:1Δ7, C18:1, total SFA and MUFA. On the other hand, significant increase was observed in the pastured birds in terms of the long chain PUFA. These changes in the fatty acid profile of the meat in the pastured chicks led to significant improvement of the dietetic quality of their meat having lower atherogenic and thrombogenic potential, and ω -6/ ω -3 ratio, as well as higher values of P/S and h/H indices. While assessing the potential of the two lines to produce high quality meat, differences in the lipid profile between the lines were observed, showing advantage of LB over BB birds. The results of this study reveal new

perspectives for further research on the rearing practices of indigenous lines in order to produce slow-growing chickens with beneficial lipid profile for a healthier human diet.

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