

Original article

## Comparison of the Fatty Acid Composition and Lipid Nutritional Indices of Meat in Two Lines of Slow-Growing Chickens and Their F1 Crosses<sup>1</sup>

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### Abstract

The study was carried out to compare the fatty acid profile and related lipid nutritional indices in the breast and thigh meat of two slow-growing lines of chickens La Belle (LB) and Bresse Gauloise (BB) and their crosses ( $\sigma$ LB $\times$  $\varphi$ BB,  $\sigma$ BB $\times$  $\varphi$ LB). The birds were reared indoors in the experimental poultry farm of the Institute of Animal Science –Kostinbrod, Bulgaria. At the age of 12 weeks, 6 male chickens of each line were slaughtered and lipid analysis of the breast and thigh meat was done. The differences in the fatty acid profile and related nutritional indices of the meat between the lines were assessed by one-way ANOVA. The fatty acid composition was affected by the crossing of the lines but to a different extent in breast and thigh meat. The highest amount of monounsaturated fatty acids (MUFA) was observed in the meat of  $\sigma$ BB $\times$  $\varphi$ LB chickens due to the significant increase of C16:1n-7 in breast and C18:1n-9 in the thigh of these birds. On the other hand, this crossbred line exhibited the lowest levels of polyunsaturated fatty acids (PUFA). Furthermore, the lowest content of MUFA was observed in the breast of the pure lines and thigh in BB and  $\sigma$ LB $\times$  $\varphi$ BB, while the highest PUFA level was determined in the breast of LB and thigh of  $\sigma$ LB $\times$  $\varphi$ BB. The differences in the meat dietetic quality described by the lipid indices showed no consistent patterns in the pure and crossbred lines depending on the type of meat. When compared with the rest, the breast meat of the LB male chickens showed significantly higher polyunsaturated/saturated fatty acids (P/S) ratio, while lowest n-6/n-3 PUFA and thrombogenic index (TI). The values of the atherogenic index (AI) and the ration between the hypo- and hypercholesterolemic fatty acids (h/H) were also improved in this line. Thigh meat however, showed best characteristics in terms of P/S and n-6/n-3 in the  $\sigma$ LB $\times$  $\varphi$ BB cross.

**Keywords:** Slow-growing chickens, Meat, Fatty acids, Lipid indices.

**Received:** 12 June 2018 \* **Accepted:** 24 September 2018 \* **DOI:** <https://doi.org/10.29329/ijjaar.2018.151.9>

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<sup>1</sup> A part of this study was presented at the International Agricultural, Biological and Life Science Conference, Edirne, Turkey, September 2-5, 2018.

## INTRODUCTION

The demand for poultry meat increases as the public awareness towards the importance of healthy human diet raises. Due to the balanced nutritional components, regular dietary intake of chicken meat has been suggested to reduce the incidence of many diseases and exert a beneficial effect on human health (Jayasena et al., 2013). Currently the needs for poultry meat are mostly supplied by products from commercial fast-growing broilers. The fast-growing genotype however is associated with declined meat quality, particularly in regard to the water-holding capacity, firmness and cohesiveness. Characterized by their superior sensory characteristics of meat, the slower-growing chicken lines have been recently drawing much more attention among the consumers demanding for traditional and higher quality poultry products. Two slow-growing lines are used in this study - the autochthonous La Belle (LB) - representative for the national gene pool in Bulgaria, as well as the old French Bresse Gauloise (BB), known for the high quality meat product in France and recently imported to Bulgaria. Increase of the potential of local slow-growing lines as meat producers can be achieved through selection and crossing, as the outcome from the latter is due to the heterosis, which is expressed in the performance of the crossbred individuals. In our previous work (Popova et al., 2017) comparing the pure LB and BB lines with their crosses, we found that the male chickens of the ♂BBx♀LB crossbred line had considerably higher live and carcass weight. Furthermore differences in the meat chemical composition were observed between lines which were dependent on the type of the meat but indicated a certain advantage of the crossbred lines, in regard to the protein content in the breast. Based on these results we continued our research to examine the effect of the crossing of both pure lines on the fatty acid composition of breast and thigh meat in male chickens. Interest in the fatty acid composition of meat stems mainly from the need to find ways to produce "healthier" meats, i.e., with higher ratios of polyunsaturated (PUFA) to saturated fatty acids and a favorable balance between n-6 and n-3 PUFAs (Wood et al., 2003). In developed countries, fatty acid composition and the total amount of saturated fatty acids (SFA) have been identified as dietary risk factors (Pascual et al., 2007).

### Material and Methods

#### *Experimental birds and rearing*

The trial was carried out in the experimental poultry farm of the Institute of Animal Science – Kostinbrod, Bulgaria with a total of 158 LB and 108 BB male chickens obtained from the parent stock of the Institute as well as their crosses (♂LBx♀BB, n=54; ♂BBx♀LB, n=53). The 1-day-old chickens were placed in a deep litter facility with a stocking density of 14 birds m<sup>2</sup> in separate pens in the same poultry house. 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%), respectively for 4 weeks 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 fatty acid composition of the diet is presented in Table 1.

**Table 1.** Fatty acid composition of the diet

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

### *Slaughtering and sampling*

At the age of 12 weeks, 6 chicks of each line were selected for slaughter based on the average live weight. 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, 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.

### *Fatty acid analysis*

Total lipids from the skinless breast and 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). 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):

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

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

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

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

### **Statistical analysis**

Data were evaluated through one way ANOVA to find significant differences due to the line of the birds in regard to fatty acid composition and lipid nutritional indices. Whenever appropriate, Tukey post hoc test ( $P<0.05$ ) was applied to compare the means of the different lines. Statistical analysis was performed using JMP v. 7 software package (JMP Version 7, SAS Institute Inc. Cary, NC).

### **Results and Discussion**

In breast and thigh meat of the pure and crossbred lines in this study we identified 12 fatty acids (Table 2 and 3). The major part of the saturated fatty acids (SFA) consisted of C16:0 and C18:0. The results of ANOVA showed significant difference among the lines in regard to the content of C18:0 in both breast ( $P<0.01$ ) and thigh meat ( $P<0.001$ ), although they did not display the same pattern. The highest content of C18:0 in the breast was observed in BB line, while in thighs both pure lines had similar levels of this fatty acid. On the other hand, the lowest content of C18:0 in both kind of meat was observed in the chickens of BB x LB line. We did not observe any significant differences in the content of C14:0 and C16:0, however, the latter tended to have the highest content in the breast of the ♂BBx♀LB birds and the lowest in the pure LB line ( $P=0.08$ ). The tendency towards highest content of palmitic acid in breast and lowest C18:0 in both breast and thigh meat might be considered a disadvantage of the ♂BBx♀LB chickens in regards to the healthier value of meat. Despite the results for the individual SFA, their total amount in the breast and thigh meat did not differ between the lines.

**Table 2.** Fatty acid composition (% FAME) and desaturase indices in the breast meat of the pure lines and their crosses

Item	La Belle (LB)	Bresse Gauloise (BB)	♂LBx♀BB	♂BBx♀LB	SEM	Sig.
C14:0	0.68	0.71	0.71	0.72	0.02	NS
C16:0	27.59	28.69	28.73	29.15	0.23	0.08
C16:1n-7	5.92a	5.65a	5.89a	7.49b	0.23	**
C18:0	7.78ab	8.71a	8.39ab	7.29b	0.18	**
C18:1n-9	34.60	34.76	35.77	37.34	0.48	NS
C18:2n-6	19.96a	19.53ab	18.75ab	16.85b	0.44	*
C18:3n-3	0.48a	0.47ab	0.40bc	0.39c	0.01	**
C20:2n-6	0.24a	0.22a	0.21a	0.14b	0.01	*
C20:3n-6	0.28a	0.22ab	0.20ab	0.14b	0.02	*
C20:4n-6	2.18a	0.94ab	0.88ab	0.45b	0.20	**
C22:5n-3	0.20a	0.08ab	0.06b	0.04b	0.01	**
C22:6n-3	0.09a	0.02ab	0.01b	0.00	0.01	*
SFA	36.05	38.11	37.83	37.16	0.30	0.05
MUFA	40.52a	40.41a	41.66ab	44.83b	0.65	*
PUFA	23.43a	21.48ab	20.51ab	18.01b	0.62	**
n-6	22.66a	20.91ab	20.04ab	17.58b	0.59	*
n-3	0.77a	0.57ab	0.47b	0.43b	0.03	**
SCD 16	0.21ab	0.20b	0.21b	0.26a	0.007	*
SCD 18	4.48ab	3.99b	4.26ab	5.12a	0.14	*

\*P<0.05; \*\*P<0.01; NS-non significant; the values in the same row connected with different letters are statistically different (P<0.05).

The MUFA of the breast and thigh meat in this study were presented by the C16:1n-7 and C18:1n-9. Significant difference (P<0.01) among the lines existed in regard to the content of C16:1 in breast as its contents were highest in ♂BBx♀LB (P<0.05). In thighs as well, this cross showed the highest content of C18:1n-9, especially in comparison with the pure BB line (P<0.05). The results corresponded to the desaturase activity described as ratios C16:1/ C16:0 and C18:1/C18:0, being highest in the ♂BBx♀LB cross. Consequently, the total MUFA differed between the lines in breast (P<0.05) and thigh (P<0.001) showing the highest content in the ♂BBx♀LB. As stated by Dal Bosco et al. (2012), MUFA in poultry are related either to endogenous synthesis or to the gut absorption from the diet. Since in this study the 4 lines of chickens were raised under the same rearing and feeding conditions, the differences in the content of MUFA could be largely attributable to the crossbreeding of the lines.

**Table 3.** Fatty acid composition (% FAME) and desaturase activity in the thigh meat of the pure lines and their crosses

Item	La Belle (LB)	Bresse Gauloise (BB)	♂LBx♀BB	♂BBx♀LB	SEM	Sig.
C14:0	1.04	1.02	0.98	1.09	0.05	NS
C16:0	30.99	30.68	27.14	29.98	0.79	NS
C16:1n-7	8.95	8.26	8.65	10.64	0.42	NS
C18:0	7.57a	7.56a	7.07ab	5.94b	0.20	***
C18:1n-9	35.55ab	32.59b	34.31ab	38.18a	0.69	*
C18:2n-6	14.98ab	18.03ab	19.58a	13.48b	0.83	*
C18:3n-3	0.30b	0.45ab	0.49a	0.28b	0.02	**
C20:2n-6	0.13	0.17	0.19	0.12	0.01	NS
C20:3n-6	0.09	0.12	0.15	0.12	0.02	NS
C20:4n-6	0.37ab	1.03ab	1.37a	0.17b	0.17	*
C22:5n-3	0.03	0.06	0.11	0.00	0.01	NS
C22:6n-3	0.00	0.03	0.02	0.00	0.008	NS
SFA	39.60	39.26	35.13	37.01	0.77	NS
MUFA	44.50ab	40.85b	42.96b	48.82a	0.81	***
PUFA	15.90ab	19.89ab	21.91a	14.17b	1.04	*
n-6	15.57ab	19.36ab	21.29a	13.89b	1.002	*
n-3	0.33b	0.54a	0.62a	0.28b	0.04	*
SCD 16	0.29a	0.27a	0.32ab	0.35b	0.01	*
SCD 18	4.70a	4.31a	4.89a	6.43b	0.21	***

\*P<0.05; \*\*P<0.01; \*\*\* P<0.001; NS-non significant; the values in the same row connected with different letters are statistically different (P<0.05).

In a study with fast-, medium and slow- growing lines, Sirri et al. (2010) found positive relationship between the content of MUFA and the intramuscular lipid content of breast and thigh meat. Previously examining the differences in the carcass quality and meat chemical composition of these pure lines and their crosses (Popova et al., 2017) we reported that the ♂BBx♀LB crossbred line exhibited higher content of intramuscular lipids when compared to the other lines, and it was especially pronounced in the thigh meat. This corresponded to the highest MUFA levels of this crossbreed that we observe d here. Furthermore, Legrand and Hermier (1992) found that the content of C16:1n-7 resulting from hepatic  $\Delta$ -9 desaturation was higher in fat chickens hence displaying higher  $\Delta$ -9 desaturase activity, which is in agreement with our results.

The content of C18:2n-6 and C18:3n-3 differed significantly between the lines, showing advantage of the pure lines when breast meat was assessed. In thighs however, the highest levels of these fatty acids were observed in the ♂LBx♀BB crossbred chickens. In both kinds of meat the ♂BBx♀LB Line showed the lowest content of C18:2n-6 and C18:3n-3. These results are in agreement with Legrand and Hermier (1992) who reported decrease in the levels of linoleic acid with increase of the fat content

in the chickens. Such relationship was also reported by Wood et al. (2008). On the other hand the lower content of  $\alpha$ -linolenic acid corresponded to the increased activity of the  $\Delta$ -9 desaturase enzyme which was observed in the ♂BBx♀LB birds. According to Kouba and Mourot (1998), and Kouba et al. (2003), the increased levels of dietary C18:3n-3 in pigs were associated with suppressed desaturase activity, which shows the inverse relationship between the deposited C18:3n-3 in the tissues and the  $\Delta$ -9 desaturase. This coincides with our results in this study in regards to the chickens of ♂BBx♀LB cross. The content of the rest of the individual PUFA in breast showed significant discrepancies between the lines and crosses which followed the same pattern and displayed the lowest levels in ♂BBx♀LB and highest in the pure LB line ( $P < 0.05$ ). In thigh meat no such results were observed except for the C20:4n-6 which had highest amounts in ♂LBx♀BB and lowest in ♂BBx♀LB. The lack to observe consistent results for the differences in the contents of the individual long chain PUFA between the lines in the breast and thigh meat suggests different action of the desaturases and elongases involved in their synthesis from the precursors C18:2n-6 and C18:3n-3 in the different kind of muscles. The differences in the total content of PUFA were determined by those of the individual PUFA and were significant in both breast ( $P < 0.01$ ) and thigh ( $P < 0.05$ ) meat.

The nutritional indices in the meat, affected to the highest extent by the differences existing between the lines, were the ratios P/S as well as n-6/n-3 (Table 4 and 5).

**Table 4.** Lipid nutritional indices in the breast meat of the pure lines and their crosses

Item	La Belle (LB)	Bresse Gauloise (BB)	♂LBx♀BB	♂BBx♀LB	SEM	Sig.
P/S	0.65a	0.56ab	0.54ab	0.48b	0.02	**
n-6/n-3	29.43b	36.68ab	42.63a	40.88a	1.49	***
AI	0.47	0.51	0.51	0.51	0.006	0.08
TI	1.06b	1.18a	1.17a	1.14ab	0.01	**
h/H	2.05	1.91	1.91	1.85	0.02	0.06

\*\* $P < 0.01$ ; \*\*\*  $P < 0.001$ ; NS-non significant; the values in the same row connected with different letters are statistically different ( $P < 0.05$ ).

**Table 5.** Lipid nutritional indices in the thigh meat of the pure lines and their crosses

Item	La Belle (LB)	Bresse Gauloise (BB)	♂LB x ♀BB	♂BB x ♀LB	SEM	Sig.
P/S	0.40ab	0.51ab	0.62a	0.38b	0.04	*
n-6/n-3	47.18a	35.83ab	34.34b	49.60a	1.82	**
AI	0.58	0.57	0.48	0.55	0.02	NS
TI	1.28	1.24	1.03	1.15	0.04	NS
h/H	1.61	1.66	2.00	1.68	0.08	NS

\*P<0.05; \*\*P<0.01; NS-non significant; the values in the same row connected with different letters are statistically different (P<0.05).

The trends observed were not consistent between the two kinds of meat. In general, the breast meat of the pure lines was characterized by better values of these nutritional indices when compared to the crossbred chickens. In thigh meat, the most favourable values of P/S and n-6/n-3 were observed respectively in the chickens of the BB line and the cross ♂LBx♀BB. In line with the above mentioned results, the TI of the breast meat displayed the lowest value in the LB birds, as they also tended to have the lowest AI (P=0.08) and the highest value of the ratio h/H (P=0.06). In thighs, these indices remained unaffected by the line of the birds. As seen from the results, the values of the atherogenic index are not much higher than the recommended maximum values of 0.5 (Ulbricht and Southgate, 1991) and the P/S ratio is above the recommended minimum of 0.4. On the other hand, the n-6/n-3 ratio is considerably higher than 4 (Simopoulos, 2004). Its values correspond to the observed differences in the n-6 and n-3 PUFA between the pure and the crossbred lines. Since the n-3 long chain PUFA are synthesised from the precursor C18:3n-3 and it is exclusively derived from the diet, various feeding strategies increasing its content in the tissues or supplementation with other long chain PUFA such as 20:5n-3, 22:6n-3 should be considered in order to lower the n-6/n-3 ratio and improve the dietetic quality of the chicken meat.

### Conclusions

The results of the study showed differences in the fatty acid profile which can be attributed to the crossing of the two slow-growing lines, but depending on the type of the meat. In line with the already found better performance in controlled microclimate, the chickens of the ♂BBx♀LB crossbreed showed the highest MUFA contents, however decreased levels of C18:0 in the breast and thigh meat. Certain advantage of the pure lines was determined in regard of the n-3 and n-6 PUFA in breast, however in the thighs higher levels of these fatty acids were observed in the ♂LBx♀BB cross. The results provide good information about the potential of the crossbred lines to produce high quality meat based on the fatty acid composition when raised in controlled conditions, however further studies are necessary to completely clarify the mechanisms leading to the differences of the fatty acid deposition in breast and thigh, as well as the rearing conditions which are best to modify the lipid profile in order to meet the requirements for a healthy diet.



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