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Chemical composition and fatty acid profile of meat from Bulgarian Grey cattle – comparison of three muscles

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Abstract

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The study aimed to compare the chemical composition and the fatty acid profile of three muscles – m. *Longissimus thoracis et lumborum* (m. LTL), m. *Semimembranosus* (m. SM) and m. *Supraspinatus* (m. SP) in male Bulgarian Grey calves. The animals were 12 months old and reared extensively on stable pasture. The three muscles differed significantly in their chemical composition. The highest protein content was determined in m. LTL, while m. SP had the highest level of intramuscular fat, moisture, and myoglobin. The analysis of the fatty acid profile showed a significant effect of the muscle type regarding the percentage of C16:0 ($P = 0.0313$), C16:1 ($P = 0.0040$), C18:0 ($P = 0.0016$), C18:2n-6 ($P = 0.0292$), C20:2n-6 ($P = 0.0029$), C20:4n-6 ($P = 0.0158$), C22:4n-6 ($P = 0.0168$), and C22:5n-3 ($P = 0.0062$), among the three muscles, m. LTL had the highest percentage of C16:0 and C18:2n-6, and the lowest percentages of C16:1n-9 and C22:4n-6. The content of C18:0 was highest in m. SP, whereas m. SM displayed the highest amounts of C20:2n-6, C20:4n-6, C20:5n-3, C22:4n-6, and C22:5n-3. There were no significant differences in AI and TI among the muscles; however, the latter tended to be higher in m. SP. In addition, the n-6/n-3 ratio values did not differ between the muscles. In m.SM and m.SP's values were within 5.22-5.26, slightly exceeding the dietary recommendations.

Keywords: Bulgarian Grey cattle; muscles; chemical composition; fatty acids; meat quality

Introduction

Meat is a crucial component of a well-balanced human diet. Recently, there have been strong debates regarding the health qualities of the so-called red meats, such as beef. According to some studies, the extreme consumption of this kind of meat is associated with increased risk of various diseases and conditions, including cancer, diabetes, and cardiovascular diseases (Wang et al., 2016; Kim et al., 2017). More recent research has shown that there is no clear link between red meat consumption and chronic health conditions (Lescinsky et al., 2022; Sanders et al., 2023). On the contrary, they are a valuable source of protein, vitamins, and microelements, including Fe, Zn, and P (Williams, 2007). The global production of beef ranks third, after chicken and pork, at 59.98 million tons for 2023 (USDA, 2024).

Along with the above-mentioned valuable nutrients, beef is a source of essential and polyunsaturated n-3 fatty acids. The latter are abundant in the pastured animals (Ponnampalam et al., 2024). Meat quality is a complex phenomenon affected by a wide range of factors, among which the breed of the animals is particularly important. Due to the specificities of their rearing practices, the indigenous cattle breeds possess unique meat traits that can significantly contribute to the production of traditional meat products (Shabtay, 2015). Bulgarian Grey Cattle is one of the two indigenous cattle breeds in Bulgaria (Neov et al., 2013). It is extensively reared on stable grazing in the regions mainly found in the mountains of Strandzha, Sakar, Central Stara Planina (Balkan Mountains), Sashtinska Sredna Gora, Rila, the Rhodopes, and in the regions of Shumen and Varna. According to Gaddini (2019), the meat of this breed is fatty and suitable for sausage production. In fact, the meat of the Bulgarian Grey is one of the two meats, together with the Eastern Balkan pig, that is used for the manufacturing of the famous traditional dry-cured sausage "Smyadovska lukanka".

Despite studies on this breed primarily focusing on its genetic profile (Hristov et al., 2014) or breeding and rearing (Gorinov and Lidji, 2011; Lidji and Gorinov, 2013), research on the nutritional and fatty acid composition of the meat in Bulgarian grey cattle is relatively scarce. Hence, this study aimed to provide information and compare the chemical composition and fatty acid profiles of three muscles (m. *Longissimus thoracis et lumborum*, m. *Semimembranosus,* and m. *Supraspinatus)* with high commercial value derived from Bulgarian Grey cattle.

Material and Methods

Experimental animals

A total of four Bulgarian Grey male calves were used in the study. The animals were reared in a farm in Krupnik village, Blagoevgrad region. The farm is located on the northeastern slopes of the Krupnishki hill, on the west side, along the Struma River, in southwestern Bulgaria. The area is characterized by permanent droughts during the summer and autumn seasons, as well as poor grass growth. The animals were reared extensively on pasture without any additional feeding with concentrate at any age period.

Slaughtering and sampling

At the age of 12 months, the calves were slaughtered in a certified abattoir. The carcasses were split in half and kept for 24 h at 4°C. The right half of each carcass was used, as m. LTL, m. SM and m. SP belonging to sirloin, round, and chuck was carefully dissected from each half and minced. Samples for analysis of proximate composition and fatty acid profile were taken, immediately frozen, and kept at –20 °C until analysis.

Chemical composition analysis

The contents of protein, fat, moisture, and ash of the studied muscles were determined according to the AOAC (2004) method. The myoglobin content was determined as described by Hornsey (1956) using a T60 UV/Visible spectrophotometer (PG Instruments).

Fatty acid profile

The extraction of intramuscular lipids was performed according to the method of Bligh and Dyer (1959) with slight modifications (Vargas-Ramella et al., 2020). Methyl esters of the fatty acids were obtained as described by Domínguez et al. (2015). The fatty acid composition was determined using a gas chromatograph (CSi 200 series, Cambridge Scientific Instruments Ltd., Ely, UK), equipped with a capillary column and hydrogen as the gas vector. The fatty acids were presented as a percentage of the methyl esters identified. Individual fatty acids were used to calculate the atherogenic and thrombogenic indices (Ulbricht and Southgate, 1991):

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

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

Statistical evaluation

The results were statistically evaluated using JMP v.7 software. One-way ANOVA was applied to assess the effect of muscle type on the examined traits. Whenever needed, the differences among means were assessed through post-hoc comparisons using the Tukey HSD test ($P < 0.05$).

Results and Discussion

Chemical composition of the muscles

The results in Table 1 showed that the type of muscle significantly affected the protein content ($P = 0.005$), fats ($P = 0.006$), moisture ($P = 0.030$), and myoglobin ($P = 0.004$). The highest protein content was detected in m. LTL, while m. SP had the highest level of intramuscular fat, moisture, and myoglobin. The content of the ash tended to be higher in m. LTL and m. SP, compared to m. SM.

The physiological functions of the different muscles in farm animals, as well as the type of fibers that predominate in them, affect the quality characteristics of the meat, including its chemical composition. The muscles in this study are part of commercially valuable cuts and have considerable discrepancies in the metabolism of their fibers. M. LTL and m. SM are composed mainly of fast glycolytic fibers (Kirchofer et al., 2002; Chaosap et al., 2021; Lebedová et al., 2021; Song et al., 2020, 2022), whereas in m. SP contains the highest percentage of slow oxidative fibers (Chaosap et al., 2021). The higher protein content in m. LTL and m.SM and the low moisture when compared to m. SP agrees with the results of Chaosap et al. (2021, 2024). However, in contrast to our data, Chaosap et al. (2024) reported a higher content

Table 1. Chemical composition of m. LTL, m. SM and m. SP of Bulgarian Grey calves

Trait	Muscle			SEM	Significance
	m. LTL	m. SM	m. SP		
Protein, %	21.34[a]	20.86[ab]	20.02[b]	0.41	P = 0.005
Fat, %	0.39[b]	0.41[b]	0.58[a]	0.06	P = 0.006
Ash, %	1.06	0.98	0.99	0.04	P = 0.057
Moisture, %	77.21[b]	77.75[ab]	78.41[a]	0.52	P = 0.030
Myoglobin, mg/g	2.77[b]	3.23[ab]	3.86[a]	0.32	P = 0.004

Means connected with different superscripts differ significantly P< 0.05
Source: Authors' own elaboration

of intramuscular fat in m. LT than m. SP in a Thai native cattle breed. Similar results were presented by Hwang et al. (2010), who found significantly higher intramuscular fat content in the longissimus muscle compared to the m. *Psoas major* contains mainly slow oxidative fibers in the Korean breed Hanwoo. Our results suggest that the intramuscular fat content of the muscles is related to the predominant type of fibers. According to Joo et al. (2017), there was a significant positive correlation between the intramuscular fat content and the number of fibers of type IA, and also a significant negative correlation between this trait and the content of type IIB fibers. We observed this regarding the higher fat content in m. SP. Hwang et al. (2010) reported considerably higher content of myoglobin in m. *Psoas major*, compared to the longissimus muscle and m.SM, which is confirmed by the higher values of this trait in m. SP with predominating slow oxidative fibers. According to Hwang et al. (2010), slow oxidative and intermediate muscle fibers contain more myoglobin, which was confirmed by a positive correlation between myoglobin content and the percentage of type I fibers.

Fatty acid profile of the muscles

A total of 18 fatty acids were identified in the muscles of the Bulgarian Grey calves. Most abundant were oleic (C18:1n-9), palmitic (C16:0), stearic (C18:0), and linoleic (C18:2n-6), which is typical for the fatty acid profile of the muscles in ruminants (Popova, 2014; Belhaj et al., 2020; Gonzales-Baron et al., 2021).

The analysis showed significant differences between the muscles in regard to the percentage of C16:0 (P = 0.0313), C16:1 (P = 0.0040), C18:0 (P = 0.0016), C18:2n-6 (P = 0.0292), C20:2n-6 (P = 0.0029), C20:4n-6 (P = 0.0158),

Table 2. Fatty acid composition (% FAME) of m. LTL, m. SM and M.SP of Bulgarian Grey calves

Fatty acids	Muscle			SEM	Significance
	m. LTL	m. SM	m. SP		
C14:0	2.75	3.04	3.44	0.65	P = 0.3576
C15:0	0.76	1.08	1.04	0.34	P = 0.3895
C16:0	21.96[a]	20.79[ab]	20.13[b]	0.81	P = 0.0313
C16:1n-9	3.42[b]	4.77[a]	4.55[a]	0.43	P = 0.0040
C17:0	1.08	0.83	0.97	0.15	P = 0.1295
C17:1	1.05	0.85	0.89	0.18	P = 0.3101
C18:0	11.72[b]	10.81[b]	13.45[a]	0.70	P = 0.0016
C18:1n-9	32.51	32.57	32.02	1.73	P = 0.8884
C18:2n-6	12.21[a]	11.29[ab]	11.21[b]	0.47	P = 0.0292
C18:3n-3	3.91	3.44	3.45	0.48	P = 0.3335
CLA	0.92	1.16	1.17	0.25	P = 0.3764
C20:2n-6	0.07[ab]	0.13[a]	0.02[b]	0.03	P = 0.0029
C20:3n-6	1.15	1.03	0.87	0.18	P = 0.1682
C20:4n-6	4.62[b]	5.41[a]	4.72[b]	0.32	P = 0.0158
C20:5n-3	0.02	0.07	ND	0.05	P = 0.1981
C22:4n-6	1.66[b]	2.39[a]	1.93[ab]	0.28	P = 0.0168
C22:5n-3	0.14[b]	0.34[a]	0.14[b]	0.07	P = 0.0062
C22:6n-3	0.05	ND	ND	0.05	-

ND-not detected; Means connected with different superscripts differ significantly P < 0.05
Source: Authors' own elaboration

C22:4n-6(P = 0.0168), and C22:5n-3 (P = 0.0062). Longissimus muscle displayed the highest percentage of C16:0 and C18:2n-6, and the lowest of C16:1n-9 and C 22:4 n-6. The content of C18:0 was highest in m. SP, whereas in m. SM we found the highest amounts of the long chain polyunsaturated fatty acids C20:2n-6, C20:4n-6, C22:4n-6 and C22:5n-3, as well as C20:5n-3. In line with our results, Chaosap et al. (2024) showed higher content in the longissimus muscle compared to the *Biceps femoris, Infraspinatus, Semimembranosus, and Supraspinatus*. On the other hand, the authors observed higher levels of C18:2n-6 in m. SM and m. SP, that contradicts to us. When studying 10 beef muscles, Hwang & Joo (2016) found higher concentrations of C20:4n-6 in m. SM, compared to m.LT and m. LL. It is worth noting that in this study, we identified long-chain polyunsaturated fatty acids (C20-C22), as their amounts varied according to the muscle type. Contrary to us, Chaosap et al. (2024) found only C22:6n-3 in five muscles of cattle of a local Thai breed. Our results are in agreement with Hoehne et al. (2012), who reported C20:4n-6; C20:2n-6; C22:5n-3 and C22:6n-3 in the *longissimus* muscle of Charolais × German Holstein bulls. The observed discrepancies among the studies may be associated with the differences in the contents of C18:2n-6 and C18:3n-3, which are precursors for the synthesis of long-chain polyunsaturated fatty acids. The content of C18:2n-6 that we measured in this study ranged from 11.21% to 12.21%, whereas the percentage of C18:3n-3 was 3.44% to 3.91%. In other studies, the contents of these fatty acids were considerably lower (Hwang & Joo, 2016; Horcada-Ibáñez et al., 2016; Joo et al., 2017; Chaosap et al., 2024).

As presented in Table 3, the fatty acid profile of the muscles showed that the total amount of saturated fatty acids was 36.55%–39.03%, followed by monounsaturated fatty acids (36.98%–38.19%) and polyunsaturated fatty acids (23.51%–25.26%).

Significant differences between the muscles were found in regard to the PUFA content (P = 0.011), which was highest in в m. SM was associated with a higher amount of n-6 PUFA in this muscle (P = 0.0016). The lowest amount of PUFA was found in m. SP corresponding to the higher amount of intramuscular fat. This suggests a higher content of neutral lipids, which augment the content of SFA and MUFA but reduce the content of PUFA (De Smet et al., 2004; Wood et al., 2004). There were no significant differences in AI and TI among the muscles; however, the latter tended to be higher in m. SP. The values of AI were in the range of 0.53 to 0.56. Bermingham et al. (2021) evaluated the fatty acid profile, phospholipids, and the atherogenic index of carcass parts, including m. LD in pastured and indoor-raised cattle. They found values of AI within the range of 0.69–1.07S. Surprisingly, the higher values of the trait were reported for the pastured animals, likely due to the decreased MUFA content. Hwang and Joo (2017) also found higher values of AI (0.60–0.84) in m. *Longisssimus lumborum* of Hanwoo cattle, American and Australian crossbreeds. The values of AI and TI that we determined in this study are close to those reported by Vázquez-Mosquera et al. (2023) in Wagyu cattle and European crossbreeds.

We did not observe significant differences in the n-6/n-3 ratio between the muscles. In this study, they ranged from 4.78 to 5.26. It has been recommended that the values of this ratio should range between 1:1 and 5:1 (Gonzales-Beccera et al., 2023), and recent studies have well demonstrated the benefits of a low n-6/n-3 value on health (Yang et al., 2016; Van Name et al., 2020). As a whole, lower AI and TI, as well as low n-6/n-3 are indicative of the high healthy value of beef. These values can be favourably altered through manipulation of the fatty acid profile of meat by applying suitable feeding and rearing strategies for cattle, aiming to increase the content of MUFA and PUFA, particularly n-3.

Table 3. Total amounts of fatty acids in m. LTL, m. SM and M.SP of Bulgarian Grey calves

Item	Musle			SEM	Significance
	m. LTL	m.SM	m.SP		
SFA	38.27	36.55	39.03	1.97	P = 0.2435
MUFA	36.98	38.19	37.46	1.80	P = 0.6450
PUFA	24.75[ab]	25.26[a]	23.51[b]	0.64	P = 0.0111
n-6	19.71[a]	20.25[a]	18.75[b]	0.39	P = 0.0016
n-3	4.12	3.85	3.59	0.49	P = 0.3743
AI	0.54	0.53	0.56	0.05	P = 0.5685
TI	0.89	0.84	0.94	0.05	P = 0.0593
n-6/n-3	4.78	5.26	5.22	0.60	P = 0.6824

SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA- polyunsaturated fatty acids, AI – atherogenic index, TI-thrombogenic index; Means connected with different superscripts differ significantly $P<0.05$

Source: Authors' own elaboration

Conclusions

The study demonstrated significant differences in the chemical composition and the fatty acid profile between m. LTL, m. SM and m. SP of calves from the Bulgarian Grey breed. Both m. LTL and m. SM had higher protein content when compared to m. SP, while the latter had higher intramuscular fat. This was associated with its less favourable fatty acid profile, showing a lower amount of PUFA. The ratio n-6/n-3 PUFA for m.SM and m.SP exceeded the dietary recommendations slightly, indicating a certain imbalance in the fatty acid profile of these muscles regarding polyunsaturated fatty acids. This could be amended through proper feeding strategies and supplementation of the animals, aiming to increase the amount of n-3 fatty acids, which warrants further study.

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