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Relationships between sarcomere length and basic composition of *infraspinatus* and *longissimus* dorsi muscle

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Abstract: The purpose of this study was to determine the relationship between sarcomere length and basic composition (water, protein, ash, collagen, and fat content including marbling) of beef muscles with fast-twitch glycolytic (*longissimus dorsi muscle*) and slow-twitch oxidative fibers (*infraspinatus muscle*) in Limousin bulls. Samples (n = 15) were obtained from federally inspected slaughter facilities, and animals used in the experiment were treated according to standard ethical norms. The shortest sarcomeres were observed for *longissimus dorsi* from striploin, and the longest for *infraspinatus* muscle. Additionally, the influence of both cut and animal was observed. In both cases of muscles, sarcomere length was positively correlated with marbling level and negatively correlated with protein content. Moreover, sarcomere length was very strongly correlated with marbling level in the case of *longissimus dorsi et lumborum*. Differences in sarcomere length were associated with cut and animal, and this correlation may partly explain differences in composition, especially in protein content.

Key words: Beef, computer image analysis, marbling, protein, sarcomere

1. Introduction

Meat quality varies to a great extent within and between animals as well as in individual muscles. This results from the fact that meat features depend on breed, genotype, sex, age, nutrition, and slaughter procedures. Individual muscles from various anatomical locations are characterized by different metabolic fiber types. *Longissimus dorsi* muscle (*LD*M) is characterized by an abundance of fast-twitch glycolytic fibers (type IIB), while *infraspinatus* muscle (*I*M) consists mostly of slow-twitch oxidative fibers (type I) (1). Several muscles are also characterized by diverse sarcomere length (2), chemical composition (3), and intramuscular fat quantity (marbling) (4). These factors influence beef tenderness, whereas sarcomere length plays a crucial role in the mechanical structure of meat (5).

It has been indicated that sarcomere length determines 16% of the variation in tenderness of *semitendinosus* muscle and 44%–55% of the variation in tenderness of *LD*M (6) when measured with the method of Warner–Brazler share force (7). At the same time, the evaluation of *LD*M tenderness with sensory methods suggests that sarcomere length explains between 14% and 38% (7) of variation in tenderness.

According to Stolowski et al. (2), sarcomere length is not affected by breed. Instead, it may depend to a large extent on the specific muscle, as sarcomere length is associated with the type of fibers dominant in the muscle. It has been indicated that type IIB fibers are characterized by a shorter sarcomere length than type I fibers (8). Li et al. (4) reported that sarcomere length increases with advancing marbling score. At the same time, it should be emphasized that until now little attention has been paid to muscles other than *LD*M and to the physiological function and metabolic types of fibers.

The purpose of this study was to determine the relationship between sarcomere length and basic composition of beef meat in muscles that are characterized by different physiological functions and metabolic types of fibers: *LD*M (fast-twitch glycolytic fibers – type IIB) and *I*M (slow-twitch oxidative fibers – type I) for U conformation class and 2–3 fat class animals.

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2. Materials and methods

2.1. Preparation of samples

The analyzed samples were obtained from the federally inspected slaughter facilities, and animals used in the experiment were treated according to standard ethical norms. This study presents preliminary results, which we plan to subsequently verify in a larger group of animals.

The samples for this study originated from 5 Limousin bulls with an average hot carcass weight of 432 ± 31 kg (388–466 kg), U conformation class, and 2–3 fat class. The animals were raised according to typical practices. They were dam-reared to the age of natural weaning (8–9 months) and subsequently raised on silage and corn. All animals were slaughtered between 25 and 27 months of age and carcasses were stored at 4 °C for 5 days postmortem. Cuts were obtained from each carcass: *IM* from the blade (slow-twitch oxidative fibers — type I) and *LDM* (fast-twitch glycolytic fibers — type IIB) as the longest muscle in beef cattle. *LDM* was divided into 2 cuts: anterior from the cube roll (*longissimus dorsi et thoracis* muscle (*LDETM*) collected from Th7–Th9 vertebrae region), and posterior from the striploin (*longissimus dorsi et lumborum* muscle (*LDELM*) collected from Th12–L4 vertebrae region). Cuts were obtained from a commercial abattoir with a limited amount of intramuscular fat tissue and connective tissue. Collected meat was stored in vacuum at 0 °C for 5 days, 2 days in carcass form and 3 days in steak form. After aging, steaks were stored in vacuum at –18 °C. For the texture analysis, the representative beef steaks were thawed in a refrigerator (Küppersbusch Hausgeräte GmbH, Germany) until an internal temperature of 2 °C was reached.

2.2. Sarcomere length

Sarcomere length values were measured according to the method of Cross et al. (9) with some modifications (10). For each cut, 5 g of representative meat samples were collected and microscopic preparations were obtained from the sucrose solution. Samples were homogenized in 30 mL of cold 0.25 M sucrose at a low speed of 5000 rpm for 60 s with a PRO 200 mechanical homogenizer (PRO Scientific Inc., USA). The evaluation of the sarcomere length was conducted using the Carl Zeiss Axio Imager M2 microscope (Carl Zeiss, Germany) with the EC Plan-Neofluar 100×/1.30 Oil Ph 3 M27 objective and AxioCamMR5 camera. Microscopic preparations were observed in the differential interference contrast (DIC) (Figure 1). Sarcomere length was measured using AxioVision Rel.4.8.2 software (Carl Zeiss, Germany). Three myofibrils were selected for every sample, and the length of 25 sarcomeres was measured for each myofibril.

2.3. Near-infrared spectroscopy analyses

Basic composition was determined with the near-infrared spectroscopy (NIR) method. In order to obtain homogeneous mixtures for each cut, 150 g of representative meat samples were collected and homogenized with a blender for 30–60 s. Samples were placed on a petri dish (90 mm in diameter) in the NIR device (NIR Flex Solids N-500, spectral range 800–2500 nm). Spectral analysis software (NIR Ware 1.1, NIR Cal 5.1) was used to measure water, fat, protein, ash, and collagen content (%).

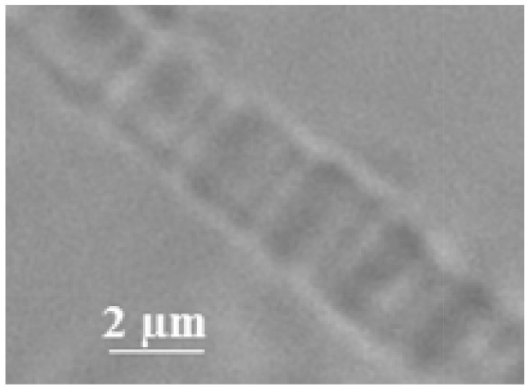

Figure 1. An example of microscopic preparation of myofibril with visible sarcomeres observed in the differential interference contrast.

2.4. Computer image analysis

Marbling (%) of meat samples was determined by computer image analysis (CIA) according to the widely applied methodology (11). After blooming for 30 min, the representative beef steaks were placed on a matte green background to ensure easier segmentation. Pictures of beef steaks were taken using a CD QImaging MicroPublisher 5.0 RTV camera under fluorescent light in standard conditions (color temperature 5400 K). Each image was captured and saved in .tif format, and the area of intramuscular fat tissue was calculated using Image-Pro Plus 7 (Figure 2).

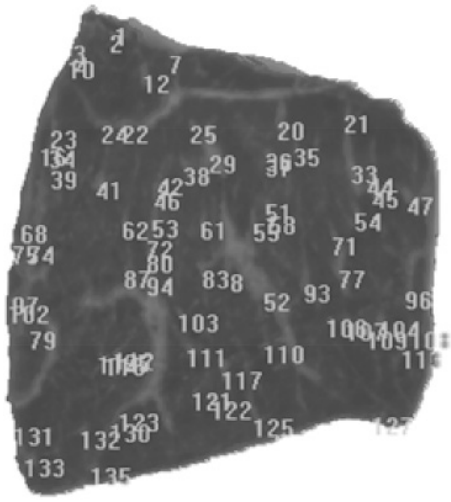

Figure 2. An example of intramuscular fat tissue (marbling) area calculation.

2.5. Statistical analysis

The W Shapiro–Wilk test was performed to verify the normality of distribution. Differences between traits were assessed using Student t-test or the Mann–Whitney U test. In order to characterize the relationships, 2-way analysis of variance (ANOVA) and post hoc tests (Scheffe test) were conducted. Analysis of correlation was carried out using Pearson's correlation or Spearman's rank correlation. Statistical analysis was conducted using Statistica software version 8.0 (StatSoft, USA), and a $P \leq 0.05$ level of significance was applied.

3. Results

Mean sarcomere length for the samples was obtained from 3 beef cuts (*IM*, *LDET*M, and *LDEL*M) of 5 bulls (Table 1) ranging from 1.82 µm (observed for *LDEL*M, the 2nd animal) to 2.87 µm (*LDET*M, the 3rd animal).

Beef characteristics from the 3 analyzed cuts, *IM*, *LDET*M, and *LDEL*M (cumulative analysis for all the animals), are presented in Table 2.

Analysis of correlation between sarcomere length and beef characteristics (cumulative analysis for all the cuts and animals) and comparison of beef characteristics between samples characterized by various sarcomere lengths is presented in Table 3.

4. Discussion

4.1. Sarcomere length for analyzed samples

The observed results are similar to results obtained in previous studies, such as that of Rhee et al. (12), where sarcomere length was 1.80 µm for *LD*M and 2.25 µm for *IM*.

ANOVA revealed that differences in sarcomere length were associated with both analyzed factors, cut (P

Table 1. Mean ± SD and median (min–max) sarcomere length for beef samples from 3 analyzed cuts and 5 analyzed animals (µm).

Animal	*Infraspinatus*		*Longissimus dorsi et thoracis*		*Longissimus dorsi et lumborum*	
	Mean ± SD	Median (min–max)	Mean ± SD	Median (min–max)	Mean ± SD	Median (min–max)
1	2.68 ± 0.10 A/a	2.68 (2.49–2.87)	2.40 ± 0.16 A/b	2.39 (2.06–2.68)	2.22 ± 0.11 A/c	2.25 (1.96–2.40)
2	2.65 ± 0.10 AB/a	2.65 (2.46–2.86)	2.72 ± 0.20 B/a	2.73 (2.21–3.05)	1.82 ± 0.11 B/b	1.79 (1.66–2.01)
3	2.50 ± 0.09 B/a	2.50 (2.33–2.65)	2.87 ± 0.15 B/b	2.88 (2.46–3.11)	2.48 ± 0.13 C/a	2.48 (2.22–2.75)
4	2.66 ± 0.14 AB/a	2.66 (2.45–2.89)	1.95 ± 0.09 C/b	1.93 (1.80–2.16)	2.08 ± 0.11 A/b	2.07 (1.81–2.31)
5	2.85 ± 0.13 C/a	2.86 (2.54–3.05)	2.33 ± 0.15 A/b	2.34 (2.03–2.61)	2.47 ± 0.11 C/b	2.46 (2.29–2.70)

For all groups: normal distribution (verification on the basis of W Shapiro–Wilk test, $P \leq 0.05$).
Mean values marked with capital letters (A, B, C) in columns and different lower case letters (a, b, c) in rows differ on the basis of post hoc Scheffe test criteria for $P \leq 0.05$.

Table 2. Mean ± SD and median (min–max) values for beef characteristics from 3 analyzed cuts (cumulative analysis for all animals): content of water, fat, protein, ash, and collagen in samples and marbling in surface.

Basic composition [%]	*Infraspinatus*		*Longissimus dorsi et thoracis*		*Longissimus dorsi et lumborum*	
	Mean ± SD	Median (min–max)	Mean ± SD	Median (min–max)	Mean ± SD	Median (min–max)
Water	74.4 ± 2.3A	75.4 (70.6–76.3)	75.8 ± 0.4A	75.7 (75.5–76.4)	75.0 ± 1.7A	75.3 (72.2–76.3)
Fat	3.4 ± 2.6A	2.8 (1.4–7.9)	1.0 ± 0.5B	1.1 (0.4–1.6)	1.4 ± 1.5AB	0.7A (0.5–4.0)[a]
Protein	19.8 ± 0.9A	19.8 (18.6–20.9)	21.7 ± 0.6B	21.9 (21.1–22.4)	22.8 ± 0.5C	22.7 (22.1–23.6)
Ash	1.1 ± 0.2A	1.2 (0.9–1.2)	1.1 ± 0.0A	1.1 (1.1–1.2)	1.1 ± 0.1A	1.1 (1.0–1.3)
Collagen	2.0 ± 0.7A	1.7A (1.6–3.3)[a]	1.3 ± 0.2B	1.2 (1.1–1.6)	1.3 ± 0.3AB	1.3 (0.9–1.7)
Marbling	3.5 ± 1.6A	3.2 (1.6–5.4)	2.6 ± 0.7A	2.9 (1.4–3.3)	1.2 ± 0.3B	1.1 (0.8–1.6)

[a]Distribution different than normal (verification on the basis of W Shapiro–Wilk test, $P \leq 0.05$).
Mean values marked with capital letters (A, B, C) in rows differ on the basis of Student t-test (normal distribution) or Mann–Whitney U test (distribution different than normal) criteria for $P \leq 0.05$.

Table 3. Analysis of correlation between sarcomere length and characteristics of beef (cumulative analysis for all cuts and animals) accompanied by comparison of beef characteristics between samples characterized by varying sarcomere lengths.

Basic composition	Correlation between sarcomere length and characteristics of beef		Comparison between samples characterized by varying sarcomere lengths				
			Mean sarcomere length lower than 2.5 μm		Mean sarcomere length higher than 2.5 μm		P-value
	P-value	R coefficient	Mean ± SD	Median (min–max)	Mean ± SD	Median (min–max)	
Water	0.771[a]	–0.0821	75.3 ± 1.3	75.6 (72.2–76.3)[a]	74.9 ± 2.1	75.5 (70.6–76.4)[a]	1.0000
Fat	0.101[a]	0.4393	1.3 ± 1.1	0.9 (0.5–4.0)[a]	2.7 ± 2.5	1.9 (0.4–7.9)[a]	0.1480
Protein	0.039*	–0.5365	22.3 ± 0.8	22.5 (21.1–23.6)	20.4 ± 1.3	20.3 (18.6–22.0)	0.0046**
Ash	0.345	0.2621	1.1 ± 0.1	1.1 (1.0–1.3)	1.1 ± 0.1	1.1 (0.8–1.2)	0.7881
Collagen	0.167[a]	0.3763	1.3 ± 0.3	1.3 (0.9–1.7)	1.7 ± 0.7	1.6 (1.1–3.3)[a]	0.1832
Marbling	0.011*	0.6353	1.7 ± 0.9	1.4 (0.8–3.3)	3.3 ± 1.4	2.9 (1.6–5.4)	0.0226**

[a]Distribution different than normal (verification on the basis of W Shapiro–Wilk test, $P \leq 0.05$).

*: $P \leq 0.05$ for Pearson's correlation (normal distribution) or Spearman's rank correlation (distribution different than normal).

**: $P \leq 0.05$ for Student t-test (normal distribution) or Mann–Whitney U test (distribution different than normal).

<0.01) and animal ($P < 0.01$). A combined effect of these factors, cut × animal, was also observed ($P < 0.01$). In paired comparisons, an analysis of sarcomere length was conducted with the same cut and animal. Sarcomeres from *LDET*M were longest in the third animal. In the case of the 4 other animals, the longest sarcomeres were observed for *I*M, which was consistent with results of other studies (13). At the same time, the shortest sarcomeres were observed in *LDEL*M. *LDET*M and *LDEL*M are parts of the same muscle, yet they have different characteristics, observed by other researchers in cases of purge loss, cooking loss, and sensory features (14). Due to the observed differences between *LDET*M and *LDEL*M, it was deemed appropriate in this study to divide the muscle into cuts and analyze the differences between the single parts. Given that the above data do indicate such differences, it can be concluded that both muscle and cut are important factors influencing sarcomere length. The results indicate differences between *LDET*M and *LDEL*M for 3 of 5 analyzed animals. However, some general conclusions may be presented for cuts. The shortest sarcomeres are observed for *LDEL*M and the longest for *I*M. These results are in general agreement with previous studies (12).

In comparisons made between animals within the same cut, differences among the paired comparisons were also observed. The longest sarcomeres were observed for animals 5 (*I*M muscles compared), 3, and 2 (*LDET*M), and 3 and 5 (*LDEL*M), respectively, while the shortest were observed for animals 4, 2, and 3 (*I*M) and 4 (*LDET*M) and 2 (*LDEL*M), respectively. Similarly, Koohmaraie (15) observed the greatest variation in sarcomere length between animals, not regarding other factors.

It should be emphasized that the longest sarcomeres (*LDET*M) and the shortest sarcomeres (*I*M) were observed in animals 2 and 3. Simultaneously, in the case of *LDEL*M, the longest sarcomeres were identified in animals 3 and 5, and the shortest in animal 2. The mean value for striploin (*LDEL*M) in the case of animal 2 was the lowest among all presented sarcomere length values, and the minimum and maximum values of sarcomere length for animal 2 were the lowest of all. This variation in sarcomere length had been reported by other authors in studies on different muscles such as *gluteus medius* and *semitendinosus* (12). However, in the case of *semitendinosus* muscle Weaver et al. found no variation in sarcomere length (16). Thus, it is essential to broaden the knowledge and understanding of all factors influencing sarcomere length.

Given that the studied animals originated from a single herd and were characterized by the same breed, sex, age, and comparable body weight, it can be suggested that features affecting sarcomere length are associated with their detailed genetics. Sarcomere length also depends on ultimate pH (17), shortening process during rigor mortis development (18), and stretch or contraction (16). However, these features were not taken into account as variables in the present study.

4.2. Basic composition of analyzed samples

In the case of certain analyzed features, such as water and ash content, no differences were observed between cuts. This was in agreement with previous reports by other researchers (19). It may be concluded that water and ash content are relatively constant within the entire carcass.

In the case of fat and collagen content, differences were observed between *I*M and *LDET*M. Both fat and collagen contents were higher in the case of *I*M (3.4% and 1.7%, respectively) than in *LDET*M (1.0% and 1.3%, respectively). In the case of *LDEL*M, fat and collagen content did not differ from the content observed for *I*M and *LDET*M. However, relationships were not the same for all features. Differences were inverse for marbling levels. Marbling for *LDEL*M (1.2%) was lower than for *I*M and *LDET*M (2.6%–3.5% for the analyzed cuts). It may be stated that differences between cuts are not identical for all features. Instead, they may depend on the construction and function of the muscle. It is generally observed that differences in muscle characteristics also occur among muscles of various types, even if animals are raised under similar production conditions (20).

The most significant differences between muscles were observed in the case of protein content. *I*M was characterized by the lowest protein content (19.8%), *LDEL*M was characterized by the highest protein content (22.8%), and the content value was intermediate for *LDET*M (21.7%). It can be concluded that protein content is the most variable feature for the analyzed cuts, in all likelihood depending on the muscle fibers.

4.3. Relationship between sarcomere length and basic composition for analyzed samples

Correlation between sarcomere length and beef characteristics is observed only in the case of protein content (analyzed with NIR) and marbling (analyzed with CIA). The comparison of beef characteristics between samples characterized by varying sarcomere lengths confirmed the obtained results. It can be stated that in the case of beef samples characterized by longer sarcomeres, lower protein content ($P = 0.039$, $R = -0.5365$ for correlation; $P = 0.005$ for comparison) and higher marbling level ($P = 0.011$, $R = 0.6353$ for correlation; $P = 0.023$ for comparison) are observed. In several studies it was proven that increase in sarcomere length is positively correlated with marbling score (4). Correlations were moderate for protein content and marbling level ($R < 0.7$), whereas sarcomere length for *LDEL*M was very strongly correlated with marbling level ($P = 0.006$; $R = 0.9692$, Pearson's correlation). It may be suggested that other unknown factors influence correlation, and the prediction of sarcomere length solely on the basis of these analyzed factors might be impossible.

In conclusion, differences in beef sarcomere length are associated with the cut and animal. The combined effect of both factors, cut × animal, was also observed. In most animals, the longest sarcomeres were observed for the *infraspinatus* muscle. In the case of *longissimus dorsi*, differences in sarcomere length between its parts (*longissimus dorsi et thoracis* and *longissimus dorsi et lumborum*) were also observed. Sarcomere length is positively correlated with content of intramuscular fat in the surface of steak and negatively correlated with content of protein. Several other beef features (content of fat, protein, collagen, and marbling) also depend on the cut, which may be partially influenced by differences in sarcomere length. Additional knowledge about sarcomere length may explain the variability of beef characteristics. Therefore, further analysis of beef sarcomere length in the case of various cuts and animals is crucial for expanding the understanding of the associations presented.

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