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Brief communication

Sarcomere length in muscles of Romosinuano Creole cattle crossed with Brahman, in Córdoba, Colombia

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ABSTRACT

Objective. To determine histologically the length of the sarcomeres (LS) in muscle samples from commercial cuts in Romosinuano by Brahman bovine bulls. **Materials and methods**. Eighteen (18) muscle cuts with four repetitions of ten bulls (approx. 24m of age and 525±28.9 kg) were analyzed for the measurement of the bands that make up the LS, by staining with hematoxylin - eosin. The number of "A bands" present at 20 µm in each of the slices were counted. **Results**. The general average of LS for the 18 cuts was 2.12 µm. The longest LS were observed in the iliopsoas (3.32 µm) and tensor fasciae latae (3.26 µm) muscles (both $p>0.05$), which were significantly longer ($p<0.05$) than those obtained for the biceps femoris (1.69 µm) and gluteus medius (1.71 µm) muscles, which were considered to have lower SL (both $p>0.05$). Conclusions. Differences in LS were found between several muscles that correspond to commercial cuts from Colombia. The differences are related to the type, location and arrangement of the muscle in the carcass during cooling. The iliopsoas muscle (sirloin or fillet), cataloged in the market as a fine cut due to its tenderness, was the one with the highest SL. This is preliminary work for a Colombian Creole cross; therefore, it is necessary to continue deepening studies that allow elucidating to what extent there is an effect of the breed and of the industrial processes (thermal treatment and disposal of the carcasses in cold chambers) on the LS; but also, about the cause/effect relationship between LS and meat tenderness.

Keywords: Bulls; Carcasses; histology; meat; muscle fibres (*Source: FAO*).

RESUMEN

Objetivo. Determinar histológicamente la longitud de los sarcómeros (LS) en muestras de músculos de cortes comerciales en toretes bovinos Romosinuano por Brahman. **Materiales y métodos.** Se analizó dieciocho (18) cortes musculares con cuatro repeticiones de diez toretes (aprox. 24m y 525±28.9 kg) para la medición de las bandas que conforman la LS, mediante tinción con hematoxilina

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– eosina. Se contó el número de "bandas A" presentes en una distancia de 20 µm en cada uno de los cortes. **Resultados.** El promedio general de LS para los 18 cortes fue de 2.12 µm. Las LS más largas se observaron en los músculos *iliopsoas* (3.32 µm) y *tensor de la fasciae latae (*3.26 µm) (ambos p>0.05), las que fueron significativamente mayores (p<0.05) que las obtenida para los músculos *biceps femoris* (1.69 µm) y *gluteus medius (*1.71 µm), los que fueron considerados de menor LS (ambos p>0.05). **Conclusiones.** Se encontraron diferencias en LS entre varios músculos que corresponden a cortes comerciales de Colombia. Las diferencias se relacionan con el tipo, ubicación y disposición del músculo en la canal durante el enfriamiento. El músculo *iliopsoas* (solomillo o filete), catalogado en el mercado como corte fino debido a su terneza, fue el de mayor LS. Este es un trabajo preliminar para un cruce criollo colombiano; por lo tanto, es necesario seguir profundizando en estudios que permitan dilucidar en qué medida existe un efecto de la raza y de los procesos industriales (tratamiento térmico y disposición de las canales en cámaras de frío) sobre la LS; pero también sobre la relación causa/efecto entre LS y terneza de la carne.

Palabras clave: Canal animal; Carne; fibras musculares; histología; torete (*Fuente: FAO*).

INTRODUCTION

Sarcomeres are contractile and functional units for the generation of basic strength in striated muscle tissue, and therefore its main functions are locomotion and the maintaining of posture in the animal, and as an important byproduct, it contributes to generating body heat (1). Anatomically, sarcomeres are limited by two Z lines. On its central level, there is an A band (anisotropic) and two I bands (isotropic). Two proteins stand out in its structure: myosin and actin (2) (Figure 1), which are designed and organized for the generation of passive and active force (3).

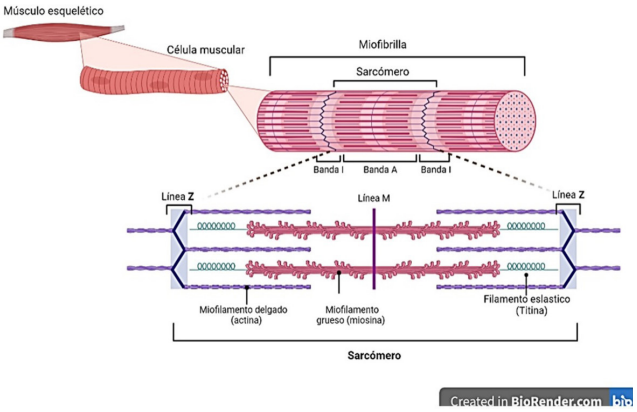

Figure 1. Anatomic Structure of the sarcomere and the arrangement of its contractile proteins. Made by the authors. Created using BioRender.

LS has a great importance regarding meat quality, since it mainly influences its tenderness, namely, the reduction or decrease in the strength of the cut (1,4,5,6) in synergy with other biochemical factors such as the final pH final, the breaking down of desmin, titin, nebulin, tropomyosin, troponin T and protein C in Line Z, mediated by calpain (7), and through the reduction of the contents and solubility of connective tissue, among other things (5,8). However, it can be affected by: the rate of post mortem reactions (pre-slaughter handling), industrial processes such as temperature treatment with extreme cold (cold shortening), the arrangement of the carcass in chambers or mechanical restriction in the muscles (1,9) diet management (6,10), anatomical location of the muscle (11), or it can even be determined by the race of the animals (12).

After the animal benefit and its transformation at the slaughterhouse, the handling of the carcass on an industrial level is an important factor, since this can determine some problems in the quality of the meat. When skeletal muscle is exposed to intense cold before the *rigor mortis* stage, a phenomenon known as *cold shortening* happens, which directly affects the length of sarcomeres, the texture and water retention of the meat; and indirectly affects the color, taste and tenderness of the meat (1), increasing from four- to fivefold the strength needed to cut the meat (13), especially when the sarcomeres are shorter than 2 µm (5). However, when the carcasses are stored in refrigerated chambers, it is important to consider the thickness of the dorsal fat layer, since this helps prevent an excessive shortening of sarcomeres during the cooling of the carcass during the *rigor mortis* stage (14). In this manner, LS is relevant since it can influence the tenderness/toughness of the meat, according to the factors mentioned above. Thus, this work had the objective of histologically determining the length of the sarcomeres in samples from different commercial cuts of Romosinuano Creole

with Cebu crossbred bovines (F1 RxC). It is worth mentioning that this study is descriptive in nature, and aimed towards the characterization of this measurement on a significant number different muscle tissue samples from F1 RxC. No data was obtained on the texture, as well as compression or resistance to cutting, which is why said information shall be supported with data from the available bibliography.

MATERIALS AND METHODS

Ethical considerations. The procedures for the use of animals were approved by the Ethical Committee for Research of Agrosavia (Act N°013). Concerning the plant of benefits or slaughterhouse, this one implements humanitarian methods of slaughter authorized by the National Institute of Surveillance of Medicine and Food Products (INVIMA) (15).

Place of study and obtaining of the samples. An evaluation was made of the muscles of ten F1 Romosinuano by Brahman bulls, raised and fattened under grazing conditions, and with a balanced ration (0.4% of live weight) of local products during the last 18 weeks until the slaughter (±24 months of age, 525±28.9 kg). The animals were slaughtered under standard commercial procedures in an exportation slaughterhouse under Decree 1500 (INVIMA registration cód. 680B/122D; 15) located in the Department of Córdoba, Colombia. After the slaughter, warm carcasses were obtained with an average weight of 283.17±22.4 Kg, with a homogeneous fat layer and an average dorsal fat layer thickness of 4.8±1.6 mm; the carcasses were transported to cooling chambers at an initial temperature between 10 to 15°C until reaching temperatures between 0 and 4°C. The pH reached at 24h was 5.69 for the loin and 5.73 for the leg. In the butchering chamber, the cuts (muscles) were obtained 48 hours after the slaughter, from left half carcasses at a temperature under 7°C. In order to evaluate the length of sarcomeres, a portion of each cut was transferred, in half an hour and at a refrigerated temperature, to the Meat Laboratory of the Turipaná Research Center, where sub-samples were immediately taken of 1.5 cm in length x 1 cm in width of the most representative muscle (4 repetitions) for each of the eighteen cuts from the ten animals (Table 1).

Table 1. Commercial name of the cut and scientific name of the most representative muscle for said cut.

Name of the Cuts	Commercial	Scientific
Fine Cuts	Tenderloin	*iliopsoas*
	Ribeye (lip on) roll	*longissimus thoraci*
	Strip Loin	*longissimus dorsi*
	Top sirloin cap	*gluteus superficiales*
Primal Cuts	Top Sirloin butt/ Rump	*gluteus medius*
	Tri-tip	*tensor fasciae latae*
	Top (inside) round	*semimembranosus*
	Knuckle (sirloin tip)	*rectus femoris*
	Eye of round	*semitendinosus*
	Rump cap	*biceps femoris (gluteobiceps)*
Sub-primal Cuts	Foreshank boneless	*extensor digitorum communis et lateralis*
	Rose meat o elephant ear	*obliquus externus abdominis*
	Shoulder Clod	*tricipitis brachii*
	Chuck (mock), Tender	*supraespinatus*
	Top blade	*infraspinatus*
	Brisket	*pectoralis profundus*
	Neck Roast	*pars cervicalis*
Retail cuts	Hind shank	*extensor digitorum lateralis et longus*

Procedures for the analysis of samples. Unmatured samples (obtained 48 hours after slaughter), were kept in a 10% formaldehyde solution for 24 hours in order to avoid lysis in the tissues. Afterwards, the formaldehyde was replaced with 70% alcohol for the conservation of each of the samples, which were then sent to an external laboratory, where a protocol was used for the processing of the samples, staining the tissues with hematoxylin - eosin (H&E). The thickness of histological cuts was 5 µm. The above was based on the protocol reported by Megías et al (16) and by Farias et al (17).

Muscle tissue samples were stained with hematoxylin - eosin (H&E), and for its analysis, the number of A bands was counted in a conveniently selected distance of 20 µm in each of the muscle samples, and the length of the sarcomere was calculated by dividing the measured distance (20 µm) by the number of A bands observed in the selected segment (17,18). The images were taken with a Nikon Eclipse Ci-L phase-contrast Microscope with an integrated Nikon DS-Ri2 digital camera, under

100x magnification, by using the NIS Elements D software. The count of the bands of sarcomeres was carried out with the Image J 1.53ª software, (19) which gave 720 readings for the entirety of the experiment.

Statistical analysis. Databases were constructed for the analysis of the information. In order to estimate the effect of the type of cut, a variance analysis was carried out through the method of least squares under a completely random design, and the significant effect of the type of cut was determined through the use of the Tukey multiple comparison test for the comparison of means with a significance of 5%. The GLM procedure of the SAS Enterprise 8.3 software was used.

RESULTS

The average length of sarcomeres obtained for each cut in the different types of muscle of the ten animals evaluated is described on Chart 2. The general mean was 2.12 µm, with a range between 1.69 and 3.32 µm. The *M. iliopsoas* (Tenderloin) and the *M. tensor fasciae latae* (Tri-Tip) were the muscles (cuts) which presented the greatest LS ($p<0.05$) when compared to the other muscles, except for the M. *pectoralis* (brisket), which had a similar LS to the muscles mentioned above. The average LS for both muscles (3.29 µm) was 55.18% higher than the general mean. On the other hand, the *M. biceps femoris* (Rump cap), presented the smallest LS, and no differences were found ($p>0.05$) with 14 muscles (Table 2). The average of these 14 muscles (1.91 µm) was 9.91% below the general mean (Figure 2).

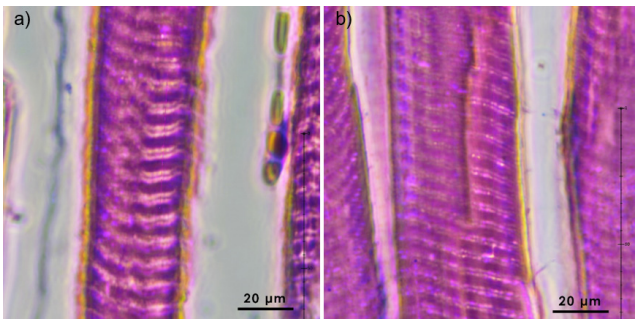

Figure 2. Muscle tissue under H&E staining for the measurement of sarcomeres. a) Tenderloin (*M. iliopsoas*). b) Chuck Tender (*M. supraespinatus*).

Table 2. Average, mínimum, máximum values and standard deviation from sarcomere length (µm) in muscles of full bovine carcasses, F1 crossbreed between Romosinuano and commercial Cebú (± 24 months of age).

Location (quarters)	Scientific name	Mean	DE	min-max.	SE
Fine cuts					
Hindquarter	*iliopsoas*	3.32 a*	0.34	2.77-3.83	0.09
Forequarter	*longissimus thoraci*	1.87 cd	0.15	1.67–2.08	0.09
Hindquarter	*longissimus dorsi*	1.90 cd	0.53	1.12-3.21	0.09
Hindquarter	*gluteus superficiales (glutebiceps)*	1.80 d	0.12	1.63–2.06	0.09
Primal Cuts					
Hindquarter	*gluteus medius*	1.71 d	0.14	1.44-1.91	0.09
Hindquarter	*tensor fasciae latae*	3.26 a	0.48	2.43-3.83	0.09
Hindquarter	*Semimembranosus*	2.05 cd	0.29	1.52-2.46	0.09
Hindquarter	*rectus femoris*	1.85 cd	0.14	1.70-2.11	0.09
Hindquarter	*Semitendinosus*	2.14 bcd	0.32	1.78-2.86	0.09
Hindquarter	*biceps femoris (gluteobiceps)*	1.69 d	0.19	1.34–1.95	0.09
Sub-Primal Cuts					
Forequarter	*extensor digitorum communis*	2.02 cd	0.32	1.71-2.59	0.09
Forequarter	*obliquus externus abdominis*	1.74 d	0.21	1.35–2.18	0.09
Forequarter	*tricipitis brachii*	2.33 bc	0.23	2.06–2.80	0.09
Forequarter	*Supraspinatus*	2.03 cd	0.37	1.58–2.77	0.09
Forequarter	*Infraspinatus*	2.13 bcd	0.32	1.74–2.86	0.09
Forequarter	*pectoralis profundus*	2.57 ab	0.32	1.82-2.98	0.09
Forequarter	*pars cervicalis*	1.97 cd	0.25	1.65–2.52	0.09
Retail Cuts					
Hindquarter	*extensor digitorum lateralis*	1.79 d	0.14	1.63-2.06	0.09

*The different letters within the column indicate significant differences. $p<0.05$

In Figure 3, it is possible to observe the structural differences in the sarcomeres of the *M. iliopsoas* muscles, also called tenderloin, which presented the greatest length (3.32 µm) when compared with the *M. biceps femoris* muscle, commercially known as Rump cap, which presented the smallest sarcomere length, with 1.69 µm, and the presence of a difference between the aforementioned cuts ($p<0.05$).

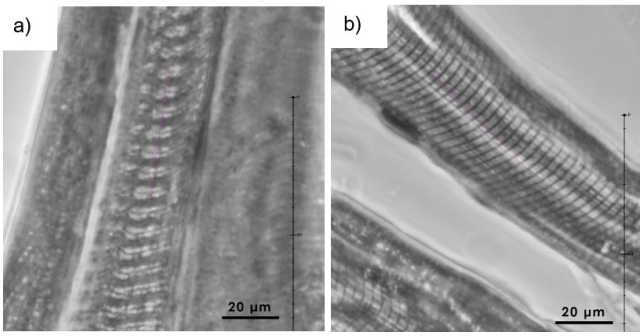

Figure 3. Structural units of the sarcomere. A) Tenderloin (*M. iliopsoas*). B) Rump cap (*M. biceps femoris*).

DISCUSSION

According to the results obtained, the average length of sarcomeres (LS) in the muscles evaluated in the F1 Romosinuano with Cebú crossbreed (F1 RxC) was 2.12 µm. As Heinemann et al (13) reported in carcasses of young Nelore bullocks sampled in refrigerator, the *semimembranosus* muscle was 0.1 µm shorter than the mean found in the present study (2.05 µm). There were similar findings with the *longissimus dorsi* muscle, which was slightly shorter (0.08 µm) than what was found for the F1 RxC crossbreed (1.90 µm). However, Guzek et al (11) reported an LS of 2.25 µm for the m *longissimus* in Bulls of the Limousin race, which is 0.35 µm higher than what was found in the F1 RxC crossbreed.

On the other hand, in the aforementioned study by Heinemann et al (13), it was found that the LS of the *bíceps femoris* was 1.88 µm, namely, 0.19 µm higher than in the F1 RxC crossbreed.

For the *longissimus thoraci* muscle, Guzek et al (11) found a mean of 2.45 µm, which was 0.58 µm higher to the one reported in the present study. Additionally, García-Torres et al (6) reported, in Retinta cattle from three different production systems, an LS of 2.73 µm for this cut, meaning, 0.86 µm higher than what was found for the F1 RxC crossbreed. Furthermore, Farias et al (17) observed a LS of 1.59 µm in fresh samples of the same muscle in adult males of the Nelore race, which was 0.28 µm lower than what was reported in this study. It is worth mentioning that in the study by Farias et al (17), after a maturation of 7 and 14 days, an increase of 1.75 and 1.66 µm in LS was observed, respectively; which suggests that in Nelore specimens, a minimum maturation time is necessary in order to increase LS. Additionally, these authors indicate that the variations in measurements of sarcomeres between one cut and another may be due to the form and anatomic location of the muscles, with the cooling speed being higher in the muscles with a smaller transversal section and located in more exposed positions in the live animal, which increases the post mortem contraction of sarcomeres.

The results of this study confirm what was indicated by said authors (17), since the variability in LS is caused mainly by the differences observed in the form (functions) and locations of the muscles in the carcass, and their relation with cold on each of them. In this case, the Tenderloin (*Iliopsoas*) and the Tri-Tip (*tensor fasciae latae*), one classified as a fine cut and the other as a primal cut, were the ones with the greatest differences above the general mean. The first one is located in the forequarters of the carcass, protected by the vertebrae, dorsal muscles and a layer of fat, which leaves it separated from a direct contact with the cold. However, the second of the aforementioned muscles, which is a primal cut, is more exposed and obtained a similar LS to the fine cut, which should be related with the level of activity of the latter and the fat layer cover, which was quite homogeneous for these carcasses. It is important to mention that no linearity was observed for the LS values and the classification of the cut, since even various sub-primal cuts were found with a greater LS than some fine cuts. Regarding this last point, Lee et al (20) evaluated the LS in five meat cuts in Bulls and steers between 24 and 30 months of age, of the Hanwoo race native to Korea, and obtained values in the muscles *pars cervicalis* (2.88 µm, sub-primal cut), *longissimus dorsi* (2.53 µm, fine cut), *rectus femoris* (2.28 µm, primal cut) and *Par pectoralis* (3.72 µm, sub-primal cut), which were superior and which were non-linear with the type of cut, in contrast with the findings of the present study. Nevertheless, the measurement of the sarcomeres for the *iliopsoas* muscle (fine cut) was lower in the Hanwoo race (2.95 µm) than in our F1 RxC. Something similar happened with the LS value observed in the *infraespinatus* muscle, which was lower (2.13 µm) to what was found by Guzek et al (11), who obtained an average of 2.67 µm for the Limousin race, with this muscle being considered a sub-primal cut.

Given that the animals in this study all have the same race, sex, similar age and body weight, it is possible to infer that the general differences in LS found regarding other studies could basically

be due to genetics and to the maturation of the samples in some studies (this study did not apply maturation). In this manner, the greatest differences found were with the Korean race Hanwoo (generally superior LS values); which decreased with tropical races such as Nelore. Another of the variables which may influence LS is pH. In the present study, only the pH of the carcass and then of the matured cuts were obtained. The pH of the carcass taken at the level of the loin and the leg had an average of 5.69 and 5.73, respectively, which are considered normal values. Due to the observational and baseline nature of this study, the evaluation of the technological processes belonging to the Company (monitoring of the temperatura and wind speed and specific location of carcasses in the chambers in relation to cooling equipment) was not considered.

In conclusion, in carcasses of F1 RxC, the *M. iliopsoas*, commercially known as tenderloin, obtained the greatest length of sarcomeres, with a quite marked difference in relation with most cuts. This is considered a fine cut due to its attributes of tenderness and quick cooking. However, no linear relation was found to correspond between LS and the type of cut (fine, primal, sub-prima, or retail cut). This means that it is possible to find sub-primal cuts with quality characteristics that are similar to those of a fine cut, since various sub-primal cuts had LS values higher than those of fine cuts.

The LS is an important indicator in order to understand the transformation of the muscle into meat during the post mortem stage, its relation with the arrangement of carcasses in cold chambers, the cooling periods and maturation on the quality of meat. Some topics that were not included in this study. Therefore, more studies are necessary in order to observe the behavior of these muscles regarding the management of cold, the position of the carcass, the maturation and tenderness, which might be of great interest for the meat industry. Likewise, animal breed holds a crucial importance, which is why a recommendation is made to expand this type of studies in order to learn more about the genetic contribution of Creole races on LS values, even without carrying out the maturation process.

Conflict of interests

The authors declare that there is no conflict of interests.

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