MEASURING BEEF SARCOMERE LENGTH BY LASER DIFFRACTION AND PHASE CONTRAST MICROSCOPY, AND ITS RELATIONSHIP WITH INSTRUMENTAL TENDERNESS

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Abstract – Tenderness has been considered the most important among the palatability attributes of meat, and therefore the main determinant of its quality. Muscle sarcomere length (SL) has been exhaustively evaluated in experiments to determine whether tenderness was affected by cold shortening. The purpose of this research was to compare the SL of *Longissimus dorsi* muscle (LD), measured by laser diffraction and phase contrast microscopy, and evaluate the relationship between sarcomere length and instrumental tenderness, measured by Warner Bratzler shear force. The conclusion reached is that laser diffraction gives lowers lengths of sarcomere when compared with phase contrast microscopy, and the higher difference is seen in shorter sarcomeres. Both methods can be used to explain beef tenderness, however tender meat is less dependent of sarcomere length.

I. INTRODUCTION

Tenderness has been considered the most important among the palatability attributes of meat, and therefore the main determinant of its quality (Huffman et al., 1996), and also the reason for dissatisfaction of up to 20% of consumers in the USA (Morgan et al., 1991). Tenderness displays wide variation among animals, breeds, muscles and cuts (Reuter, Wulf, & Maddock, 2002).

Variations in the myofibrillar protein structure can have significant effects on tenderness. Muscle sarcomere length (SL) has been exhaustively evaluated in experiments to determine whether tenderness was affected by cold shortening (Pflanzer & de Felicio, 2009).

Two methods are very much cited in the literature for measuring SL, laser diffraction and phase contrast microscopy. However, there are only a few scientific works that present a comparison between measurements obtained with these methods, being difficult to explain some low values obtained with laser diffraction in the absence of cold shortening (Cross et al., 1981).

The purpose of this research was to compare the SL of *Longissimus dorsi* muscle (LD), measured by laser diffraction and phase contrast microscopy, and evaluate the relationship between sarcomere length and instrumental tenderness, measured by Warner Bratzler shear force.

II. MATERIALS AND METHODS

Three experiments were carried out (Table 1). The LD muscle (12th rib), was evaluated for ultimate pH (pHu), cooking loss (CL), Warner Bratzler shear force (WBSF) and sarcomere length (SL). The pHu and SL were measured on fresh samples 4 days post mortem, while CL and WBSF were evaluated after 14 days of aging.

Table 1. Description of experiments

Description	Trial 1	Trial 2	Trial 3
n	7	13	16
Breed	Angus x Nellore (F1)	Angus x Nellore (F1)	Bos indicus (BI)
Age (mo)	18	18	24-36
Gender	Heifer	Bull	Bull

For WBSF and CL, steaks of approximately 2.5 cm thick were cooked in a conventional electrical oven (170ºC), and the steaks' internal temperatures were individually monitored. Steaks were removed from the oven when they reached the internal temperature of 71ºC. Cooked steaks were weighed, packed in plastic bags, and chilled at 4ºC for 24 h. Six 1.27 cm cores per steak were removed, parallel to the muscle fibers (AMSA, 1995). Each core was sheared by a texture meter TA-XT 2i, fitted with a 1 mm thick Warner–Bratzler blade.

SL, by phase contrast microscopy, was carried out according Culler, Parrish, Smith, & Cross

(1978). A suspension of myofibrils from each sample was prepared by homogenization with ice-cold buffer of pH 7.0. Drops of this myofibril suspension were placed on microscope slides with cover slips, and examined under a phase contrast microscope (Nikon, Model Eclipse CI-L) utilizing lenses of 100x and a 10x ocular, in oil immersion. Images of myofibrils displaying better-defined structures were captured, and five sarcomeres of each were measured using digital image analysis software (Nikon, NIS-Elements).

SL, by laser diffraction, was carried out according Koolmees et al. (1986). Samples (2 x 1cm) were fixed in glutaraldehyde (4 hours) and small fibers bundles were passed through the helium-neon laser (Red HeNe Laser, 633 nm, 2.0 mW).

The statistical analyses were performed by ANOVA one way, and the results expressed as mean±SEM. The means were tested by Tukey test ($P < 0.05$) using the Statistica 7.0 software (Statsoft, 2005).

III. RESULTS AND DISCUSSION

There were no difference ($P > 0.05$) for pH and CL between trials evaluated, however it was found difference ($P < 0.05$) for WBSF. Trial 3 had the higher WBSF, and no difference was found between trials 1 and 2 (Table 2).

Table 2. Mean and SEM of pH, cooking loss and shear force by trials

Description	Trial 1	Trial 2	Trial 3
pH	5.50±0.03	5.55±0.06	5.58±0.03
CL (%)	24.45±1.37	22.60±0.74	23.80±0.72
WBSF (kg)	3.47±0.22[b]	3.71±0.13[b]	6.12±0.39[a]

[ab] Within a factor, means without a common superscript letter differ (P<0.05)

Sarcomere length was not affected by trials ($P > 0.05$), however sarcomere measured by laser was shorter ($P < 0.05$) than sarcomere measured by microscopy. In general (all trials), by laser, SL ranged from 1.31 to 2.22µm, while by microscopy ranged from 1.80 to 2.32µm (Table 3).

It was found a significant correlation ($r = -0.71$; $P < 0.001$) between SL and WBSF just in trial 3, independent of method used for SL (Table 4). It could be explained because the higher WBSF found in trial 3. Trials 1 and 2 had tender samples, probably because genetic type cattle, and SL had little effect on tenderness.

There was a positive correlation ($P < 0.001$) from SL measured by microscopy and laser for all trials evaluated, and the correlation coefficient were 0.87, 0.91 and 0.80 for trials 1, 2 and 3, respectively (Table 4).

Table 3. Descriptive analysis of sarcomere length by trials

	Trial 1	Trial 2	Trial 3
Laser			
Mean±SEM	1.78±0.05[b]	1.80±0.05[b]	1.71±0.04[b]
Minimum	1.57	1.48	1.31
Maximun	1.99	2.22	2.03
Microscopy			
Mean±SEM	2.03±0.04[a]	2.06±0.03[a]	2.04±0.03[a]
Minimum	1.87	1.81	1.80
Maximun	2.15	2.32	2.17

[ab] Within a trial, means without a common superscript letter differ (P<0.05)

Table 4. Correlation coefficients among shear force and sarcomere length

	Trial 1	Trial 2	Trial 3
Laser x WBSF	0.114338	-0.026835	-0.706449*
Microscopy x WBSF	-0.036408	0.021096	-0.706176*
Laser x Microscopy	0.870128*	0.908136*	0.803480*

* P < 0.001

Figure 1 shows the tendency line of trials 1, 2 and 3 when plotting data from SL measured by laser and microscopy. It can be seen that for all trials the lines have a similar tendency.

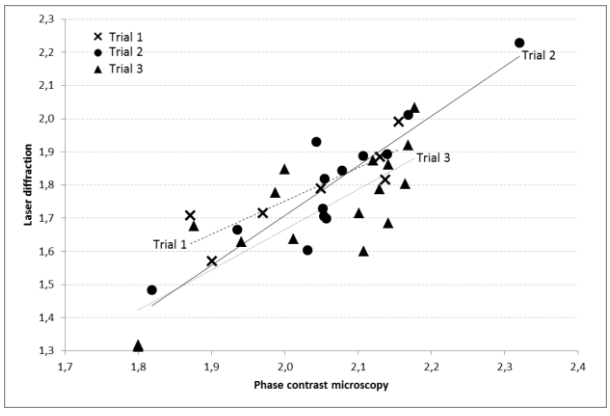

Figure 1. Tendency line of trials 1, 2 and 3 for sarcomere length measure by laser diffraction and phase contrast microscopy.

Regarding the regression procedures, all trials were plotted together (Figure 2), and it was

found a linear trend for SL measured by microscopy and laser (R^2 = 0.69; SLlaser = 1.89*SLmicroscopy – 0.86).
In Figure 2 it is possible recognize that there is a higher difference of SL in the lower side of the length range. In longer sarcomeres, the differences from methods are smaller.

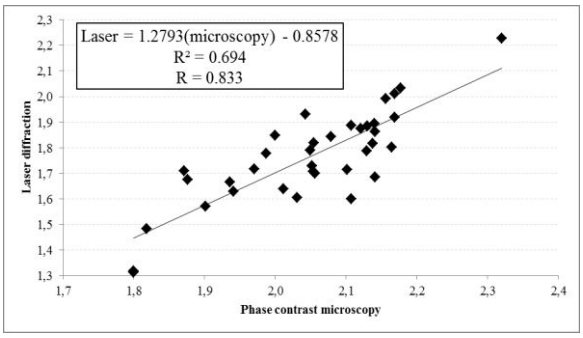

Figure 2. Correlation and regression coefficients, and prediction equation for sarcomere length measured by laser diffraction and phase contrast microscopy.

IV. CONCLUSION

The conclusion reached is that laser diffraction gives lowers lengths of sarcomere when compared with phase contrast microscopy, and the higher difference is seen in shorter sarcomeres. Both methods can be used to explain beef tenderness, however tender meat is less dependent of sarcomere length.

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