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Full Length Research Paper

Characterization of *rigor mortis* of *longissimus dorsi* and *triceps brachii* muscles of male cattle carcasses

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In this work, six bovine carcasses butchered in a slaughterhouse in Rio de Janeiro State, Brazil (SIE 504) were studied and temperature, pH, sarcomere length in different periods after slaughter (1, 5, 8, 12, 15 and 24 h) of the *longissimus dorsi* (LD) and *triceps brachii* (TB) muscles as well as the shear force (meat tenderness) and colour were evaluated, aiming at characterizing the *rigor mortis* in the meat during industrial processing. Data statistic treatment demonstrated that carcass temperature and pH decreased gradually during the industrial chilling. The chilly room temperature varied from 10.2 to 4.0°C, the mean initial temperature of *longissimus dorsi* was 34.03°C and the final one was 7.12°C; the mean initial temperature of *Triceps brachii* was 39.00°C and the final one was 6.42°C. The mean initial pH of *Longissimus dorsi* was 6.47 and the final one was 5.46; the mean initial pH of *Triceps brachii* was 6.66 and the final one was 5.54. The smallest sarcomere size obtained in both muscles occurred at 12 h post mortem, and the sarcomere lengths were 1.44 and 1.41 µm, respectively. As for the colour parameters, the *b value presented higher correlation with the ultimate pH. The absence of cold shortening and the non-occurrence of dark firm and dry (DFD) meat indicate better quality of the meat analyzed.**

Key words: Bovine carcass, muscles, *rigor mortis.*

INTRODUCTION

In the last few years, Brazil has become the world's main exporting country of bovine meat (Brazilian National Beef Cattle Council, 2011). On the other hand, when new markets are conquered, the demands of consumers incited by the publicity over quality meat give rise to a greater demand by the retail market for carcasses with desired qualitative (colour, tenderness and sensory) and quantitative (better yield of) characteristics from the slaughter and meat processing plants. Therefore, the search for quality meat from younger animals and with good carcass finishing is increasingly higher (Faria, 2005).

Meat can be considered as a noble food for mankind. Besides the nutritional aspect, the pH and temperature reduction during the onset of *rigor mortis* in carcasses of meat animals have a direct influence on the meat quality. The *rigor mortis* speed is mainly controlled by the muscle's glycogen reserve, pH and temperature. The sarcomere length is directly related to the *rigor mortis* onset and meat tenderness. The *rigor mortis* process can be characterized by measuring the sarcomere length using an optical microscope, and by following the pH decline. This measurement undergoes changes during this event with progressive reduction of the sarcomere length until the resolution of the *rigor mortis* (Wheeler and

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Koohmaraie, 1994).

An important physical property of the meat is the tenderness (Swatland, 1984), which is strongly influenced by the pH and temperature (Marsh et al., 1981). Colour is another significant physical component in beef and consumers use it as a quality and freshness indicator (Maria et al., 2003). Fresh meat has a bright red colour due to the presence of oxymyoglobin which results from the combination of myoglobin with oxygen. Under low oxygen pressure in the meat, the oxygen link to the heme ring tends to dissociate (Dias Correia, 1976).

Thus, the purpose of this work was to evaluate the *rigor mortis* process on the *longissimus dorsi* (strip loin) and the *triceps brachii* (shoulder clod) muscles of six bovine carcasses during industrial chilling and which contribute to information regarding the quality of cattle meat.

MATERIALS AND METHODS

Animals and muscle samples

Six Nelore-breed male cattle were randomly chosen during the *ante mortem* inspection and slaughtered at a slaughterhouse under State Inspection Service (SIE 504) in Campos dos Goytacazes, Rio de Janeiro State. The stunning procedures conducted followed the Stunning Methods Technical Regulation for Humane Slaughter of Meat Animals (Brazilian Ministry of Agriculture, Livestock and Supply, 2000), and the slaughter followed the standards described in the regulation of industrial and sanitary inspection of animal products (Brazilian Ministry of Agriculture, Livestock and Supply, 1997). After the bleeding procedure, the carcasses were dressed (removal of head, feet and skin), eviscerated, cut, washed and chilled.

Temperature and pH

The left half-carcass were identified and taken to the chilling room, where their temperatures (in °C) were measured directly in the *longissimus dorsi* (LD) and *triceps brachii* (TB) (5 cm into the LD and TB muscles) using a digital thermometer with a metal rod, and the pH of the LD and TB was measured by a Handylab 1-Schott pHmeter using a homogeneous solution of 10 g of the sample in 100 ml of distilled water at time intervals of 1, 5, 8, 12, 15 and 24 h after bleeding (Silva et al., 1999).

Colour

Samples of LD (from the 6th thoracic to the 7th thoracic vertebrae) and TB were collected after 24 h postmortem from the left half-carcass of each animal and vacuum packaged. The colour components *L** (lightness), *a** (redness), *b** (yellowness) were measured instrumentally on the freshly cut surface of the raw LD and TB samples after they were allowed to bloom for 30 min. A portable Spectrophotometer (Model CM-508d, Minolta Corporation Instrument Systems Division, Ramsey, NJ, USA) using a standard illuminant D65 and 10-degree standard observer was used for all measurements. Four replicates of each loin eye sample were made.

Shear force

For shear force measurement, samples of LD (from the 6th thoracic to the 7th thoracic vertebrae) and TB were collected after 24 h postmortem from the left half-carcass of each animal, then vacuum packaged and aged for 6 days at 0 ± 1°C. Warner-Bratzler meat shear force was assessed on samples of approximately 250 g, which, after being unpacked, were removed from their bags and heated in a water bath at 75°C until their internal temperature reached 70°C. This internal temperature was monitored using a digital thermometer placed in the geometric center of each sample. After cooking, the meat was cooled down to room temperature, and samples measuring 1.27 cm in diameter and 7.0 cm in length were taken parallel to the muscle fiber direction with a stainless steel cylinder and sent for measurement. Shear force was measured using a Warner-Bratzler Shear device (Model 3000, Electronics Manufacturing Company, Manhattan, KS, USA) according to Kerth et al. (1995). Seven replicates of each sample were made.

Sarcomere length

Samples were collected from each carcass at the same muscles and time intervals mentioned above, in order to determine the sarcomere length. Samples of LD and TB measuring 2.5 cm in length, 1.5 cm thickness and 0.5 cm in width were excised from the left half-carcass of each animal and kept still by double metallic clamps placed on each end of the muscle fragment, so as to minimize the effect of sarcomere shortening. The samples were identified with a tag tied to the double clamps and fixed in a 10% buffered formalin solution. After that, the fragments were taken away from the double claws with the aid of a scalpel, and submitted to the usual microscope slide preparation techniques at the State University of North Fluminense, Food Technology Laboratory. After formalin fixation, the samples were cleaved, dehydrated, cleared, embedded in paraffin and cut into 5-µm thick sections in a Leica – RM 2145 semi-automatic microtome. The histological cuts were stained with Harris Hematoxylin and counterstained with Eosin (Behmer et al., 1976).

The sarcomere length was measured with an Olympus BH-2 light optical microscope using an oil-immersion lens (1000x) and an ocular lens with a millimeter scale, according to Sloss and Kemp (1978). This method is based on the count of 10 consecutive sarcomeres of ten different myofibrils and the sarcomere measurement is done using a millimeter ocular lens fitted with a 10 micrometers (µm) scale. The average of the values found was multiplied by a (0.8) correction factor related to the ocular lens and the immersion objective lens. Conventional optical microscopy, through the use of fixation with a 10% buffered formalin solution and a dying process, was used for the study of muscle and sarcomere length (Abreu, 1984; Almeida, 1993).

Statistical analysis

Statistical analysis regarding the *rigor mortis* process showed the behavior of the temperature, pH and sarcomere length of six repetitions, over the time intervals (1, 5, 8, 12, 15 and 24 h after bleeding), separately, for the *longissimus dorsi* and the *triceps brachii* muscles through the analysis of variance (ANOVA) of repeated measurements at a 5% significance level (SAS, 2001). A completely randomized design was used for the analysis of the shear force data using analysis of variance. Linear correlation among the results of ultimate pH, *L**, *a**, *b**, shear force (24 h) and sarcomere length was tested.

RESULTS AND DISCUSSION

The mean temperature values (°C) with respect to the *post mortem* time period (1, 5, 8, 12, 15 and 24 h) (Table

Table 1. Mean and standard deviation (X ± s) for temperatures (°C) and pH values of *longissimus dorsi* (LD) and *triceps brachii* (TB) muscles from six half bovine carcasses, at six *post mortem* time intervals (1, 5, 8, 12, 15 and 24 h) during chilling.

Analysis	Sample	Time post mortem (h)					
		1	5	8	12	15	24
Temperature (°C)	LD	34.03 (1.50)^{a}	19.72 (0.37)^{b}	12.33 (1.02)^{c}	12.23 (0.50)^{c}	7.18 (2.36)^{d}	7.12 (1.34)^{d}
	TB	39.00 (0.95)^{a}	26.18 (1.38)^{b}	22.28 (0.84)^{c}	16.75 (1.47)^{d}	15.90 (1.32)^{d}	6.42 (0.54)^{e}
pH	LD	6.47 (0.21)^{a}	6.20 (0.09)^{b}	5.98 (0.14)^{c}	5.61 (0.13)^{d}	5.58 (0.16)^{d}	5.46 (0.05)^{e}
	TB	6.66 (0.20)^{a}	6.26 (0.17)^{b}	6.08 (0.16)^{b}	5.78 (0.20)^{c}	5.66 (0.08)^{c}	5.54 (0.14)^{d}

*Mean values in the same line followed by different superscripts vary significantly according to Tukey test ($p<0.05$).

Table 2. Mean and standard deviation (X±s) for sarcomere length of *longissimus dorsi* (LD) and *triceps brachii* (TB) muscles from 6 half bovine carcasses, at six *post mortem* time intervals (1, 5, 8, 12, 15 and 24 h) during chilling.

Sample	Number	Time post mortem (h)					
		1	5	8	12	15	24
LD	6	2.34 (0.02)^{a}	2.08 (0.6)^{b}	2.04 (0.02)^{b}	1.80 (0.03)^{c}	1.94 (0.03)^{d}	1.97 (0.05)^{d}
TB	6	2.30 (0.03)^{a}	2.02 (0.04)^{b}	1.98 (0.03)^{b}	1.75 (0.04)^{c}	1.91 (0.02)^{d}	1.94 (0.02)^{d}

*Mean values in the same line followed by different superscripts vary significantly according to Tukey test ($p<0.05$).

1) for the *longissimus dorsi* muscle were: 34.03°C (1 h), 12.23°C (12 h) and 7.12°C (24 h); and for the *triceps brachii* muscle were: 39.00°C (1 h), 16.75°C (12 h) and 6.42°C (24 h). Statistical analysis of the data showed significant difference ($p<0.05$) regarding the temperature values at time intervals of 1 and 5 h, and 5 and 8 h for both muscles.

Mean pH values for the *longissimus dorsi* and *triceps brachii* muscles were 6.47 ± 0.21 and 6.66 ± 0.20 in the 1st hour, 6.20 ± 0.09 and 6.26 ± 0.17 in the 5th hour, 5.98 ± 0.14 and 6.08 ± 0.16 in the 8th hour, 5.61 ± 0.13 and 5.78 ± 0.20 in the 12th hour, 5.58 ± 0.16 and 5.66 ± 0.08 in the 15th hour and 5.46 ± 0.05 and 5.54 ± 0.14 in the 24th hour, respectively. Tukey's test comparisons showed no significant difference ($p>0.05$) between the mean pH values for the *longissimus dorsi* muscle at time intervals of 12 and 15 h and for the *triceps brachii* muscle at intervals of 5 and 8 h, 12 and 15 h after bleeding (Table 1). The mean pH values obtained for this study agree with those observed by Van de Water et al. (2003), who observed final average pH values of 5.46 and 5.53 for the *longissimus dorsi* muscle.

Chilling room temperatures between 1 and 7°C are the ideal range to promote a normal *rigor mortis* process and better meat tenderness (Tornberg et al., 2000). Meat can be classified according to its pH at 24 h as: normal, for a pH range of 5.5 to 5.8; moderate DFD, 5.8 < pH < 6.2 and DFD for pH > 6.2 (Silva et al., 1999). DFD meat is a problem caused by chronic stress before slaughter which uses up the glycogen levels. There is evidence that the main inducing factor for DFD meat is inadequate handling before slaughtering, which leads to animal's physical exhaustion (Roça, 2001).

The pH/temperature window was one of the initial specifications for the Meat Standards Australia (MSA) 'carcass pathways' grading scheme (Thompson, 2002). At low muscle temperatures, extensive shortening occurred and the subsequent increased toughness was termed 'cold shortening'. At high muscle temperatures, some shortening also occurred in some cases (but not all), leading to increased toughness (Simmons et al., 1997). This effect was termed 'heat shortening' (Simmons et al., 1996). These studies led to the development of the MSA pH/temperature window, whereby electrical inputs during processing were managed to achieve a pH/temperature relationship of greater than pH 6 for muscle temperatures higher than 35°C, and a pH of less than 6 for muscle temperatures lower than 12°C (Thompson, 2002).

The development of the *rigor mortis* process in meat animals shows different sarcomere lengths during the onset of the biochemical process. Therefore, when the structural changes in the muscles during the *rigor mortis* process were studied, the following mean values were found for the sarcomere length in *longissimus dorsi* and *triceps brachii* muscles: 2.34 and 2.30 µm, respectively, at the 1st hour after slaughter (Table 2).

Maximum sarcomere shortening occurred at the 12th hour for both muscles; *longissimus dorsi* (1.80 µm) and *triceps brachii* (1.75 µm). According to the sarcomere length mean values for the *longissimus dorsi* and *triceps brachii* muscles shown in Table 2, the interval from the

Table 3. Mean and standard deviation (X±s) for shear force of *longissimus dorsi* (LD) and *triceps brachii* (TB) muscles from 6 half bovine carcasses.

Sample	Number	Shear force (kg)
Longissimus dorsi (LD)	6	$2.39^{A} \pm 0.20$
Triceps brachii (TB)	6	$4.04^{B} \pm 0.65$

*Mean values in the same column followed by different superscripts vary significantly according to Tukey test ($p<0.05$).

1st hour to the 12th hour showed that there was a decrease in sarcomere length mainly due to the overlap between thick and thin filaments.

Statistical analysis did not show significant difference regarding the mean sarcomere length values for the *longissimus dorsi* and *triceps brachii* muscles at the time periods of 5 and 8 h, 12 and 24 h. At the 12th hour, the *rigor mortis* in both muscles had the lowest mean of sarcomere length due to the permanent cross bridges formed between myosin and actin.

The mean value for the sarcomere length at 12 h *post mortem* for the *longissimus dorsi* muscle was 1.80 µm, and at time 24 h *post mortem*, it was 1.97 µm, which is similar to the mean values of 1.77 and 1.94 µm at 12 and 24 h *post mortem*, respectively, found for *longissimus dorsi* by Ferreira et al. (2006). Mc Keith et al. (1985) also found similar mean value of 2.10 µm at 24 h *post mortem*. However, Hwang et al. (2004), while studying the effect of temperature, observed a sarcomere length of 1.52 µm at 24 h after the slaughter of carcasses stored at 5°C. Geesink et al. (2003), while studying the effect of clenbuterol administration on meat quality, observed a sarcomere length of 1.51 µm at 24 h after the slaughter of the control.

The mean value for the sarcomere length at 12 h *post mortem* for the *triceps brachii* muscle was 1.75 µm, and at time 24 h *post mortem*, it was 1.94 µm, which is similar to the mean values of 1.74 and 2.07 µm at 14 and 24 h *post mortem*, respectively, found for *triceps brachii* by King et al. (2003). However, Mc Keith et al. (1985) observed a sarcomere length of 2.55 µm at 24 h *post mortem*.

At the time interval of 12 and 24 h for both muscles, the action of endogenous proteases causes muscular proteolysis which brings about an increase in the sarcomere length due to the denaturation of myofibrillar proteins until the resolution of the *rigor mortis*. For Jaarsveld et al. (1997) and Koohmaraie et al. (1987), the *post-mortem* modification, characterized by myofibril degradation and fragmentation, is indispensable to confer tenderness to the meat.

Muscular proteolysis seems to be the main contributor to the meat tenderness process, which can be promoted by the endogenous and exogenous proteases. There are two systems among the endogenous proteases: Ca^{+2} dependent proteases (CDP), stored in the sarcoplasmatic reticulum, such as the calpains and cathepsins in the lysosome, and responsible for the changes during the period of resolution of the *rigor mortis*. Cooperation between the two systems has been suggested (Quali, 1992).

Mean shear force values (kg) for the *longissimus dorsi* and *triceps brachii* muscles were 2.39 ± 0.20 and 4.04 ± 0.65, respectively. Tukey's test comparisons showed significant difference ($p<0.05$) between the mean force values for the *longissimus dorsi* muscle and *triceps brachii* (Table 3). Shackleford et al. (1997) reported that carcass was classified as "tender," "intermediate" or "tough" if its *longissimus* shear value at 1 or 2 days postmortem was <6 kg, 6 to 9 kg, or >9 kg, respectively.

In this study, the *triceps brachii* muscle showed the highest ultimate pH and shear force. Purchas (1990) and Purchas and Aungsupakorn (1993) noted that a pH increase from 5.5 to 6.2 caused the toughening (increase of shear force) of meat, and suggested that this was partially due to the shortening of the sarcomeres.

Yu and Lee (1986) reported that the highest shear force and pH at 24 h are due to the fact that high pH is not considered optimum to the lysosomal enzymes, neither to the calcium-dependent proteases, thus keeping the Z-lines of sarcomeres preserved.

Data regarding the correlation among the variables (ultimate pH, sarcomere length, L^*, a^* and b^*) are given in Table 4. There was a high ($p<0.05$) linear inverse correlation between the ultimate pH and the sarcomere length ($r = -0.81$). This result agrees with those found by Shackelford et al. (1994), who observed the highest linear inverse correlation between the ultimate pH and the sarcomere length in carcasses stimulated with low voltage. In other words, as the ultimate pH increased, the sarcomere length decreased (as in the DFD meats). Similar results were reported by Purchas (1990) and Purchas and Aungsupakorn (1993), where a pH increase from 5.5 to 6.2 was accompanied by a sarcomere length decrease from 1.64 to 1.51 µm.

In the present work, a linear inverse correlation ($r = -0.57$) between the shear force (24 h postmortem) and sarcomere length was observed, but it was not significant. On the other hand, Smulders et al. (1990) observed a smaller, but significant inverse correlation between these two variables. However, when they sorted the carcasses according to the pH decline (based on the pH determination 3 h postmortem), there was a high inverse correlation ($r = -0.80$) for the carcasses that

Table 4. Correlation coefficients among the ultimate pH and shear force, sarcomere length, *L**, *a** and *b** and between shear force and sarcomere length in LD muscle.

Parameter	Shear force	Sarcomere length	*L**	*a**	*b**
Ultimate pH	0.66	-0.81*	-0.49	-0.51	-0.69*
Shear force	–	-0.57	-0.60	0.57	-0.04

¤* $p < 0.05$.

suffered a slow glycolysis (pH_3<6.3) and a negligible correlation (r = -0.12) for the carcasses that suffered fast glycolysis. Nevertheless, Shackelford et al. (1994) reported a smaller and not significant (p>0.05) inverse correlation in both types of carcasses glycolysis. They found that in slow and fast glycolysis carcasses, the correlations were r = -0.17 and r = -0.27, respectively. According to Koohmaraie (1996), the toughening that occurs on the first 24 h postmortem is due to sarcomere shortening. The correlation obtained by this author (r = -0.52) was similar to our findings. Furthermore, Yu and Lee (1986) found an even higher correlation coefficient (r = -0.76) in their studies.

As for the ultimate pH and shear force (24 h postmortem) variables, there was a positive linear correlation (r = 0.66). In other words, when the ultimate pH increased, the shear force also increased. The same was observed by Purchas (1990) and Purchas and Aungsupakorn (1993).

All three-colour parameters were negatively correlated with the ultimate pH. In this work, the *L** value (r = -0.49) was the worst indicator of the muscle ultimate pH when compared with either *a** (r = -0.51) or *b** (r = -0.69) values. This agrees with Wulf et al. (1997) findings. The *b** value was considered the best predictor of muscle ultimate pH on low voltage stimulated carcasses. Similar results were reported by Wulf and Wise (1999) for normal pH meat; there was a correlation among the *L**, *a** and *b** values and the ultimate pH of r = -0.27, -0.42, -0.46, respectively; and for DFD meat, a correlation of values r = -0.17, -0.66, -0.67, respectively. These results show that as the ultimate pH increased, the colour values decreased; therefore, the meat became darker.

The correlation among the shear force (24 h postmortem) and *L**, *a** and *b** values was not significant (p > 0.05) and the lowest correlation was observed with the *b** value. On the other hand, Wulf et al. (1997) found the highest correlation with the *b** value (p < 0.05), but they evaluated the shear force at six postmortem aging times (1, 4, 7, 14, 21 and 35 days post mortem). According to Wulf et al. (1997) and Fiems et al. (2000), the *L** value was negatively and significantly correlated with the shear force. In this study, we obtained similar results, but it was not significant.

Conclusions

The cattle carcass temperature fell when the industrial chilling occurred according to defined standards. The pH decline followed the development of the *rigor mortis* process for both muscles studied until the 24th hour after bleeding. Maximum contraction during *rigor mortis*, characterized by a shorter sarcomere length was detected at the 12th hour for the *longissimus dorsi* and *triceps brachii* muscles. The shear force in both muscles was considered to be acceptable and as for the colour parameters, the *b** value presented the larger correlation with the ultimate pH.

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REFERENCES

Abreu RL (1984). Correlation between time, temperature and pH with the onset of *rigor mortis* in beef carcass. Master Degree Dissertation. Vet. Med. College, Federal Fluminense Univ. Niterói, Rio de Janeiro, Brazil.

Almeida WM (1993). Influence of the low-voltage electrical stimulation of bleeding, pH and sarcomere length muscle beef (*Bos indicus*), during the industrial chilling. Master Degree Dissertation. Vet. Med. College, Federal Fluminense Uni. Niterói, Rio de Janeiro, Brazil.

Behmer AO, Benez SM, Bernard DM (1976). Manual techniques for normal and pathological histology. São Paulo, Brazil: EDART, p. 239.

Brazilian Ministry of Agriculture, Livestock and Supply (1997). Brazilian Secretariat of Agriculture and Livestock. Brazilian National Department of Agricultural Protection. Regulation of Industrial and Sanitary Inspection of Animal Products (Approved by Decree n°. 30.691 of March 29, 1952, modified by Decrees n^{os}. 1.255 of June 25, 1962, 1.236 of September 02, 1994, n°. 1.812 of February 08, 1996 and n°. 2.244 of June 05, 1997). DIPOA-MAPA, Brasília-DF, p. 241.

Brazilian Ministry of Agriculture, Livestock and Supply (2000). Brazilian Secretariat of Agriculture and Livestock. Stunning Methods Technical Regulation for Humane Slaughter of Meat Animals. Normative Instruction n°. 3, of January 17, 2000 (approved by Ministry Ordinance n°. 574, of December 8, 1998, process n°. 21000.003895/99-17).

Brazilian National Beef Cattle Council (2011). Beef Cattle Datasheet. Corporate website. Available at: <http://www.cnpc.org.br>. Accessed on: October 27.

Dias Correia AA (1976). Biochemistry of meat. Lisboa, Portugal: Foundation Calouste Gulbenkian. p. 822.

Faria MH (2005). Maturation of Beef Meat. Paper prepared during the Postgraduate Course in Animal Science, State University "Júlio de Mesquita Filho", Botucatu *Campus*, São Paulo, Brazil. Available at: <

http://dgta.fca.unesp.br/carnes/materialparadownload.php>. Accessed on: October 27, 2011.
Ferreira GB, Andrade CL, Costa F, Freitas MQ, Silva TJP, Santos IF (2006). Effects of transport time and rest period on the quality of electrically stimulated male cattle carcasses. Meat Sci. 74: 459-466.
Fiems LO, De Campeneere S, De Smet S, Van de Voorde D, Vanacker JM, Boucqué CV (2000). Relationship between fat depots in carcasses of beef bulls and effect on meat colour and tenderness. Meat Sci. 56: 41-47.
Geesink GH, Smulders FJ, Van Laack HL (2003). Effects on meat quality of the use of clenbuterol in veal calves. J. Anim. Sci. 71: 1161-1170.
Hwang IH, Park BY, Cho SH, Lee JM (2004). Effects of muscle shortening and proteolysis on Warner-Bratzler shear force in beef *Longissimus* and *Semitendinosus.* Meat Sci. 68: 497-505.
Jaarsveld FPV, Naude RJ, Oelofsen W (1997). The effects of CA ions, EDTA and storage time on myofibrillar protein degradation, levels of Ca^{2+} dependent proteases and cathepsins B, H, L and D of ostrich skeletal muscle. Meat Sci. 45(4): 517-529.
Kerth CR, Miller MF, Ransey CB (1995). Improvement of beef tenderness and quality traits with calcium chloride injection in beef loins 48 h *post mortem*. J. Food Sci. 73(3): 750-756.
King DA, Dikeman ME, Wheeler TL (2003). Chilling and cooking rate effects on some myofibrillar determinants of tenderness of beef. J. Anim. Sci. 81: 1473-1481.
Koohmaraie M (1996). The biochemical factors regulating the toughening and tenderization processes of meat. Meat Sci. 43: 193-201.
Koohmaraie M, Seideman SC, Schollmeyer JE, Duston TR, Crouse JD (1987). Effect of *post mortem storage on* Ca^{+2} dependent proteases, their inhibitor and myofibril fragmentation. Meat Sci. 19: 187-196.
Maria GA, Villarroel M, Sañudo C, Olleta JL, Gebresenbet G (2003). Effect of transport time and ageing on aspects of beef quality. Meat Sci. 63(4): 1335-1340.
Marsh BB, Lochner JV, Takahashi G, Kragness DD (1981). Effects of early *post-mortem* pH and temperature on beef tenderness. Meat Sci. 5(6): 479-483.
Mc Keith FK, Devol DL, Miles RS (1985). Chemical and sensory properties of thirteen major beef muscles. J. Food Sci. 50: 869-872.
Purchas RW (1990). An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. Meat Sci. 27(2): 129-140.
Purchas RW, Aungsupakorn R (1993). Further investigations into the relationship between ultimate pH and tenderness for beef samples from bulls and steers. Meat Sci. 34(2): 163-178.
Quali A (1992). Proteolytic and physicochemical mechanisms involved in meat texture development. Biochemie, 74: 251-265.
Roça RO (2001). Post-mortem Modifications. Technical paper. Agroindustry Management and Technology Department, State University "Júlio de Mesquita Filho", Botucatu *Campus*, São Paulo. Available at: < http://dgta.fca.unesp.br/carnes/ materialparadownload.php>. Accessed on: October 27, 2011.
SAS (2001), INSTITUTE SAS. SAS User's guide statistics. Cary, pp. 959.
Shackelford SD, Koohmaraie M, Savell JW (1994). Evaluation of *Longissimus dorsi* muscle pH at three hours *post mortem* as a predictor of beef tenderness. Meat Sci. 37(2): 195-204.
Shackelford SD, Wheeler TL, Koohmaraie M (1997). Tenderness classification of beef: evaluation of beef *Longissimus* shear force at 1 or 2 days *post mortem* as a predictor of aged beef tenderness. J. Anim. Sci. 75: 2417-2422.
Silva JA, Patarata L, Martins C (1999). Influence of ultimate pH on bovine meat tenderness during ageing. Meat Sci. 52(4): 453-459.
Simmons NJ, Cairney JM, Daly CC (1997). Effect of pre-rigor temperature and muscle prestraint on the biophysical properties of meat tenderness. 43rd Int. Congr. Meat Sci. Technol. Auckland, N. Zealand. 43: 608-609.
Simmons NJ, Singh K, Dobbie, PM, Devine CE (1996). The effect of pre-rigor holding temperature on calpain and calpastatin activity and meat tenderness. 42nd Int. Congr. Meat Sci. Technol. Lillehammer, Norway. 42: 414-415.
Sloss MWBS, Kemp RLAB (1978). Veterinary clinical parasitology. 5. ed. Iowa, USA: Iowa State University Press. p. 247.
Smulders FJM, Marsh BB, Swartz DR, Russel RL, Hoenecke ME (1990). Beef tenderness and sarcomere length. Meat Sci. 27(4): 349-363.
Swatland HJ (1984). Structure and properties of meat. Zaragoza, Sapin: Acribia S.A. p. 26.
Thompson J (2002). Managing meat tenderness. Meat Sci. 62(3): 295-308.
Tornberg E, Wahlgren M, Brøndum J (2000). Pre-rigor conditions in beef under varying temperature an pH falls studied with rigometer, NMR an NIR. Food Chem. 69: 407-418.
Van de Water G, Verjans F, Geers R (2003). The effects of short distance transport under commercial conditions on the physiology of slaughter calves, pH and colour profiles of veal. Livestock Prod. Sci. 82: 171-179.
Wheeler TL, Koohmaraie M (1994). Pre-rigor and post-rigor changes in tenderness of ovine *Longissimus* muscle. J. Anim. Sci. 72: 1232-1238.
Wulf DM, O'Connor SF, Tatum JD, Smith GC (1997). Using objectives measures of muscle colour to predict beef *Longissimus* tenderness. J. Anim. Sci. 75(3): 684-692.
Wulf DM, Wise JW (1999). Measuring muscle colour on beef carcasses using the *L**, *a**, *b** colour space. J. Anim. Sci. 77(9): 2418–2427.
Yu LP, Lee B (1986). Effects of *post mortem* pH and temperature on bovine muscle and meat tenderness. J. Food Sci. 51(3): 774-780.